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(54) Title: METHODS AND COMPOSITIONS OF MATTER CONCERNING APRIL/G70, BCMA, BLYS/AGP-3, AND TACI

(57) Abstract: This invention concerns interactions among APRIL/G70, AGP-3/BLYS, BCMA, and TACI and related methods of use and compositions of matter. It has been found that (1) sAPRIL/G70 binds to the cell-surface receptors BCMA and TACI on T and B lymphoma cells, resulting in stimulation of proliferation of primary human and mouse B and T cells both *in vitro* and *in vivo*; (2) APRIL competes with AGP3's binding to TACI and BCMA; (3) sBCMA inhibits APRIL and AGP3 binding to its receptors; (4) sBCMA ameliorates T cell dependent and T cell independent humoral immune responses *in vivo*; (5) sTACI inhibits APRIL and AGP3 binding to its receptors and ameliorates T cell dependent and T cell independent humoral immune responses *in vivo*; and (6) BCMA exhibits similarity with TACI within a single cysteine rich domain located N-terminal to a potential transmembrane domain. These discoveries provides a strategy for development of therapeutics for treatment of autoimmune diseases, and cancer, for prevention of transplant rejection. Disease states and disease parameters associated with APRIL and AGP-3 may be affected by modulation of BCMA or TACI; disease states and parameters associated with TACI can be affected by modulation of APRIL; disease states and parameters can be affected by modulation of any of TACI, BCMA, APRIL and AGP-3 by a single therapeutic agent or two or more therapeutic agents together.

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METHODS AND COMPOSITIONS OF MATTER CONCERNING APRIL/G70, BCMA, BLYS/AGP-3, AND TACI

This application claims the benefit of U.S. Provisional Application
5 Serial No. 60/204,039, filed May 12, 2000 and U.S. Provisional Application
Serial No. 60/214,591, filed June 27, 2000, which are incorporated herein
by reference.

Field of the Invention

10 The present invention relates to proteins that are involved in
inflammation and immunomodulation, survival, or activation or in
lymphoproliferative disorders or other cancers. The invention further
relates to proteins related to the tumor necrosis factor (TNF)/nerve
growth factor (NGF) superfamily and related nucleic acids, expression
15 vectors, host cells, and binding assays. The specification also describes
compositions and methods for the treatment of immune-related and
inflammatory, autoimmune and other immune-related diseases or
disorders, such as rheumatoid arthritis (RA), Crohn's disease (CD), lupus,
and graft versus host disease (GvHD) as well as for treatment of
20 lymphoproliferative diseases and other cancers.

Background of the invention

After years of study in necrosis of tumors, tumor necrosis factors
(TNFs) α and β were finally cloned in 1984. The ensuing years witnessed
25 the emergence of a superfamily of TNF cytokines, including fas ligand
(FasL), CD27 ligand (CD27L), CD30 ligand (CD30L), CD40 ligand
(CD40L), TNF-related apoptosis-inducing ligand (TRAIL, also designated
AGP-1), osteoprotegerin binding protein (OPG-BP or OPG ligand), 4-1BB
ligand, LIGHT, APRIL, and TALL-1. Smith *et al.* (1994), *Cell*, 76: 959-962;

Lacey *et al.* (1998), *Cell*, 93: 165-176; Chichepotiche *et al.* (1997), *J. Biol. Chem.*, 272: 32401-32410; Mauri *et al.* (1998), *Immunity*, 8: 21-30; Hahne *et al.* (1998), *J. Exp. Med.*, 188: 1185-90; Shu *et al.* (1999), *J. Leukocyte Biology*, 65: 680-3. This family is unified by its structure, particularly at the C-terminus. In addition, most members known to date are expressed in immune compartments, although some members are also expressed in other tissues or organs, as well. Smith *et al.* (1994), *Cell* 76: 959-62. All ligand members, with the exception of LT- α , are type II transmembrane proteins, characterized by a conserved 150 amino acid region within C-terminal extracellular domain. Though restricted to only 20-25% identity, the conserved 150 amino acid domain folds into a characteristic β -pleated sheet sandwich and trimerizes. This conserved region can be proteolytically released, thus generating a soluble functional form. Banner *et al.* (1993), *Cell*, 73: 431-445.

Many members within this ligand family are expressed in lymphoid enriched tissues and play important roles in the immune system development and modulation. Smith *et al.* (1994). For example, TNF α is mainly synthesized by macrophages and is an important mediator for inflammatory responses and immune defenses. Tracey & Cerami (1994), *Annu. Rev. Med.*, 45: 491-503. Fas-L, predominantly expressed in activated T cell, modulates TCR-mediated apoptosis of thymocytes. Nagata, S. & Suda, T. (1995) *Immunology Today*, 16:39-43; Castrim *et al.* (1996), *Immunity*, 5:617-27. CD40L, also expressed by activated T cells, provides an essential signal for B cell survival, proliferation and immunoglobulin isotype switching. Noelle (1996), *Immunity*, 4: 415-9.

The cognate receptors for most of the TNF ligand family members have been identified. These receptors share characteristic multiple cysteine-rich repeats within their extracellular domains, and do not possess catalytic motifs within cytoplasmic regions. Smith *et al.* (1994).

The receptors signal through direct interactions with death domain proteins (e.g. TRADD, FADD, and RIP) or with the TRAF proteins (e.g. TRAF2, TRAF3, TRAF5, and TRAF6), triggering divergent and overlapping signaling pathways, e.g. apoptosis, NF- κ B activation, or JNK activation. Wallach *et al.* (1999), Annual Review of Immunology 17: 331-67. These signaling events lead to cell death, proliferation, activation or differentiation. The expression profile of each receptor member varies. For example, TNFR1 is expressed on a broad spectrum of tissues and cells, whereas the cell surface receptor of OPGL is mainly restricted to the osteoclasts. Hsu *et al.* (1999) Proc. Natl. Acad. Sci. USA, 96:3540-5. Such proteins are believed to play a role in inflammatory and immune processes, suggesting their usefulness in treating autoimmune and inflammatory disorders.

A number of research groups have recently identified TNF family ligands with the same or substantially similar sequence, but they have not identified the associated receptor. The ligand has been variously named neutrokin- α (WO 98/18921, published May 7, 1998), 63954 (WO 98/27114, published June 25, 1998), TL5 (EP 869 180, published October 7, 1998), NTN-2 (WO 98/55620 and WO 98/55621, published December 10, 1998), TNRL1-alpha (WO 9911791, published March 11, 1999), kay ligand (WO99/12964, published March 18, 1999), and AGP-3 (U.S. Prov. App. Nos. 60/119,906, filed February 12, 1999 and 60/166,271, filed November 18, 1999, respectively). Each of these references is hereby incorporated by reference. Hereinafter, this protein sequence is referred to as "AGP-3."

A recent paper has identified two previously known proteins as receptors for AGP-3. Gross *et al.* (2000), Nature 404: 995-9. The first receptor was previously identified as a lymphocyte surface receptor named Transmembrane Activator and CAML Interactor (TACI). See WO 98/39361, published September 11, 1998, and von Bulow & Bram (1997),

Science, 278:138-140, each of which is hereby incorporated by reference in its entirety. According to these references, TACI binds an intracellular cyclophilin ligand designated CAML, which modulates the calcium signaling pathway in lymphocytes.

5 The second receptor identified for AGP-3 is the so-called B cell maturation protein (BCMA). The human BCMA gene was discovered by molecular analysis of a t(4;16) translocation, which characteristic of a human T cell lymphoma. Laabi et al. (1993), EMBO J. 11: 3897-3904. BCMA mRNA was reported to be found mainly in lymphoid tissues. Human
10 BCMA cDNA encodes a 184 amino acids protein (185 residues for the mouse), and the literature reports no obvious similarity with any known protein or motif, and its function remained unknown. The protein was reported to reside in the Golgi apparatus (Gras et al. (1995), Intl. Immunol. 7: 1093-1106). Recent speculation suggested that BCMA may be a distant
15 member of the TNFR super family. Madry et al. (1998), Intl. Immunol. 10: 1693-1702.

A ligand called APRIL or G70 is a TNF family ligand that remains without a receptor reported in the literature. According to the literature, APRIL is associated with prostate cancer, breast cancer, Alzheimer's
20 disease, immune disorders, inflammatory disorders, and gestational abnormalities. See WO 99/00518 (June 26, 1997); WO 99/11791 (Sept. 5, 1997); WO 99/12965 (Sept. 12, 1997); EP 911 633 (Oct. 8, 1997); EP 919 620 (Nov. 26, 1997); WO 99/28462 (Dec. 3, 1997); WO 99/33980 (Dec. 30, 1997); WO 99/35170 (Jan. 5, 1998); and Hahne et al. (1998), J. Exp. Med. 188:
25 1185-90. (Each of the foregoing references is hereby incorporated by reference in its entirety.) A recent paper described APRIL isoforms and suggested that APRIL causes cell death. Kelly et al. (2000), Cancer Res. 60: 1021-7. The art would benefit from identification of a receptor for APRIL and a clarification of its activity.

Summary of the Invention

It has now been found that sG70 (APRIL) binds to cell-surface receptors on T and B lymphoma cells resulting in stimulation of proliferation of primary human and mouse B and T cells both in vitro and in vivo. It has now been found that BCMA and TACI are cell-surface receptors for APRIL. It has also been found that APRIL competes with AGP3's binding to TACI and BCMA. Furthermore it is shown here that sBCMA inhibits APRIL and AGP3 binding to its receptors. sBCMA ameliorates T cell dependent and T cell independent humoral immune responses in vivo. In addition it has now been found that sTACI inhibits APRIL and AGP3 binding to its receptors and ameliorates T cell dependent and T cell independent humoral immune responses in vivo. In addition it has now been found that sBCMA reduces lymphoma and colon carcinoma cell tumor growth *in vivo*. It has also now been found that sBCMA increases survival and reduces incidence of proteinurea, and development of anti-dsDNA antibodies in an animal model of lupus. It has also been found that BCMA exhibits similarity with TACI within a single cysteine rich domain located N-terminal to a potential transmembrane domain. It has also been found that APRIL stimulates B cell growth and immunoglobulin production in vitro and in vivo. Furthermore, treatment with a blocking anti-APRIL antibody ameliorates generation of antigen specific immunoglobulin, suggesting that endogenous APRIL is required for humoral immunity in vivo. This invention concerns novel methods of use and compositions of matter that exploit these discoveries. The discoveries provides a strategy for development of therapeutics for treatment of autoimmune diseases, and cancer, for prevention of transplant rejection.

These discoveries show that activity, disease states, and disease parameters associated with APRIL and AGP-3 may be affected by modulation of BCMA. Likewise, disease states and disease parameters

associated with TACI can be affected by modulation of APRIL. Further, such disease states and disease parameters can be affected by modulation of any of TACI, BCMA, APRIL and AGP-3 together. This discovery further suggests molecules and methods of treatment by which more than one of TACI, BCMA, APRIL, and AGP-3 may be modulated by a single molecule.

Description of the Figures

Figure 1 shows the sequence of human APRIL (SEQ ID NOS: 1 and 2)

10 Start and stop codons are underlined.

Figures 2A and 2B show the DNA and amino acid sequences of mouse APRIL/G70 (SEQ ID NOS: 3 and 4, respectively). Start and stop codons are underlined. The amino acid sequence of FLAG-tagged soluble mouse APRIL (SEQ ID NO: 19) is also provided.

15 Figure 3 shows an alignment of human (SEQ ID NO: 22) and mouse (SEQ ID NO: 23) APRIL. The middle line of each row shows the consensus sequence (SEQ ID NO: 24).

Figure 4 shows that G70/APRIL is a potent stimulator for B and T cell lymphoma. Figure 4A shows dose-dependent stimulation of proliferation of Jurkat cells (human leukemic T cells), Raji (human Burkitt lymphoma) and K562 cells (human chronic myelogenous leukemia cells).. The proliferation of cells was determined by incubating 3×10^4 cells/well in 100 μ l medium with indicated concentration of recombinant sG70/APRIL and phosphate-buffered saline (PBS, no ligand) as a control. After 48 hours, the number of viable cells were measured by Celltiter 96 AQ proliferation assay (Promega, Madison, WI). In Figure 4B, U937 cell (monocyte-like leukemia cells), NIH/3T3 (mouse embryo cell line) and 293 (transformed human primary embryonal kidney cell line) did not respond to sG70/APRIL stimulation.

Figures 5A and 5B show FACS analysis of G70/APRIL receptor binding. G70/APRIL receptor expression was assessed on indicated cell line using anti-Flag monoclonal antibody followed by FITC-conjugated goat antibody to mouse IgG. A anti-mouse CD16/CD32 monoclonal antibody (Fc Block) was used to block non-specific binding to cells.

Figure 6 shows the effect of sG70/APRIL on human peripheral blood B cell, T cell and granulocyte proliferation. Human peripheral T cell (CD4+ and CD8+) , B cells and granulocyte were purified from three different donors by using RosetteSep cocktail antibodies (Stem cell Tech. Vancouver). Purified cells were cultured in tissue culture-treated plastic wells(Becton-Dickinson , Lincoln park, NJ) for 6 Days in RPMI-1640 medium supplemented with 10% fetal calf serum ,2 mM L-glutamine and 2-ME (50uM) in the present different concentration of sG70/APRIL. For the B cell proliferation assay, plastic wells were coated with purified mouse anti-human Ig M monoclonal antibody (3 µg/ml, Pharmingen, San Diego, CA). The positive control for T cell stimulation is IL-2.

Figure 7 shows the effect of G70/APRIL on murine T-and B-cell proliferation in vitro. T-and B-cells from the spleens of C57B1 mice were purified by selection through a murine T-cell and B-cell enrichment columns. 1×10^5 cells per well were cultured in the absence or presence of various G70/APRIL for 48 hours, pulsed during the last 18 hours with 0.5 µCi ^3H thymidine and harvested to count the incorporated radioactivity.

Figure 8 shows the effect of G70/APRIL on murine T cell proliferation costimulated though anti-CD28 antibody. T-cells from the spleens of C57B1 mice were purified by selection through a murine T-cell enrichment column. 1×10^5 T-cells per well were treated with G70/APRIL in the absence or presence of subliminal concentration of anti-CD28 antibody (0.9 µg/ml) for 48 hours, pulsed during the last 18 hours with 0.5 µCi ^3H thymidine and harvested to count the incorporated radioactivity.

Figure 9 shows the effect of G70/APRIL on murine T cell proliferation costimulated through anti-CD3 antibody. T-cells from the spleens of C57B1 mice were purified by selection through a murine T-cell enrichment column. 1×10^5 T-cells per well were treated with G70/APRIL in the absence or presence of subliminal concentration of anti-CD3 antibody ($0.9 \mu\text{g/ml}$) for 48 hours, pulsed during the last 18 hours with $0.5 \mu\text{Ci } ^3\text{H}$ thymidine and harvested to count the incorporated radioactivity. Table 1 shows FACS analysis of spleen (Table 1A), and mesenteric lymph nodes (Table 1B) after in vivo systemic administration of TNF family members. Several members of TNF family have been tested in vivo, each group have 5 mice (BDF-1, 8 weeks of age, Dose: 1 mg/kg/day 0.2 ml for 5 days). Spleen, thymus and mesenteric lymph nodes from three mice of each group have been isolated for FACS analysis using a panel of T cell and B cell surface mark antibodies. Results of FACS analysis have been summarized as following tables.

Figures 10A and 10B show the sequence of human BCMA (SEQ ID NO: 5). BCMA's extracellular domain (SEQ ID NO: 6) extends from aa 1 to aa 51 and is identified by arrows. The cysteine-rich consensus region (SEQ ID NO: 7, described further hereinafter) is shown in boldface. The transmembrane region (SEQ ID NO: 8) is underlined. huBCMA-Fc (SEQ ID NO: 9). mBCMA-Fc (SEQ ID NO: 10).

Figure 11 shows an alignment of human BCMA amino acid sequence and murine BCMA amino acid sequence (SEQ ID NO: 11). The human sequence is shown on the top line, the murine on the bottom line in each row. The human-murine consensus sequence (SEQ ID NO: 12) appears as the middle line of each row. A "+" in the consensus sequence indicates a conservative substitution. The cysteine-rich portion of the consensus sequence (SEQ ID NO: 13) appears in boldface.

Figures 12A and 12B show the sequence of hTACI (SEQ ID NO: 14). TACI's extracellular domain (SEQ ID NO: 15) extends from aa 1 to aa 166. The cysteine-rich consensus region (SEQ ID NO: 16) is shown in boldface, and the transmembrane region (SEQ ID NO: 17) is underlined. hTACI-Fc
 5 (SEQ ID NO: 18).

Figure 13 shows an alignment of cysteine rich extracellular regions of human TACI and human BCMA. The BCMA cysteine rich consensus region (SEQ ID NO: 20) appears as the top line, the TACI cysteine rich consensus region (SEQ ID NO: 21) appears as the bottom line of each row.
 10 Conserved amino acid residues are indicated by a vertical bar (|). Related amino acid residues are indicated with a colon (:).

Figures 14A, 14B and 14C show soluble mouse G70/APRIL binding to 293 cells expressing the BCMA gene. Human 293 cells transfected with the pmBCMA and pcDNA3 vectors were incubated with G70/APRIL-
 15 Flag, followed by FITC-conjugated anti-Flag antibody staining for FACS analysis. A. 293 cells transfected with pcDNA3 vector only. B. 293 cells transfected with antisense pmBCMA vector. C. 293 cells transfected with sense pmBCMA vector.

Table 2 shows BLACore analysis of the stoichiometric binding
 20 kinetics of APRIL and AGP-3 to BCMA and TACI. Flag-APRIL specifically binds to murine and human BCMA with affinities of 0.25 nM and 0.29 nM, respectively, and to human TACI with an affinity of 1.48 nM. Also a longer version of Flag-tagged APRIL (aa 50-240) binds to BCMA and TACI with high affinity similar to that of Fc-AGP-3 (Table 2). In
 25 separate experiments, we determined that neither APRIL nor AGP-3 bind to OPG and also that TNF α , OPGL, LIGHT, TWEAK, and TRAIL do not bind to BCMA or TACI. Hence, APRIL and AGP-3 specifically bind to both BCMA and TACI with high affinity.

Figure 15 shows G70/APRIL binding to 293 cells expressing the hTACI gene. Human 293 cells transfected with the phTACI and pcDNA3 vectors were incubated with G70/APRIL-Flag, followed by FITC-conjugated anti-Flag antibody staining for FACS analysis. In Figure 15A, 293 cells were transfected with phTACI vector. In Figure 15B, 293 cells were transfected with pcDNA3 vector only.

Figure 16 shows G70/APRIL completely blocks AGP3 binding to its receptor. Mouse B lymphoma cells A20 were stained with AGP3-Fc or plus 10 fold excess G70/APRIL, CD40 ligand, TRAIL ligand and Tweak. After washing 3 times, cells were incubated with FITC-conjugated goat anti-human IgG-Fc secondary antibody. In Figure 16A, 10 fold G70/APRIL completely blocked AGP3 binding to A20 cells. In Figures 16B,C and D, 10 fold CD40 ligand, Tweak and TRAIL do not have that effect on AGP3 binding.

Figure 17 shows that soluble TACI receptor (sTACI) binding competes with G70/APRIL binding to A20 cells. A20 cells were incubated with G70/APRIL or at same time plus 10 fold soluble TACI, TRAIL R2 and TRAIL R3 receptor, followed by FITC-conjugated anti-Flag antibody staining for FACS analysis. In Figure 17A, soluble TACI receptor partially competed in binding G70/APRIL binding to A20 cells. In Figures 17B and C, soluble TRAIL R2 and TRAIL R3 receptors did not interfere with G70/APRIL binding.

Figure 18 shows that soluble human BCMA-Fc receptor fusion protein (shBCMA-Fc) and soluble human TACI-Fc receptor fusion protein (shTACI-Fc) completely blocks soluble human AGP-3-Fc receptor fusion protein (shAGP3-Fc) binding to A20 cells. A20 cells were incubated shAGP3-Fc or at same time plus 10 fold shBCMA-Fc or shTACI-Fc followed by FITC-conjugated anti-Flag antibody staining for FACS analysis.

Figure 19A shows shBCMA-Fc completely blocks shAGP3-Fc binding to A20 cells. A20 cells were incubated with shAGP3-Fc with or without 10 fold soluble hBCMA-Fc followed by FITC-conjugated anti-Flag antibody staining for FACS analysis . Figure 19B shows that shBCMA-Fc blocks soluble murine APRIL (smAPRIL) binding to A20 cells. A20 cells were incubated with smAPRIL with or without 10 fold shBCMA-Fc followed by FITC-conjugated anti-Flag antibody staining for FACS analysis .

Figure 20 shows serum levels of anti-KLH IgG and IgM and anti-Pneumovax IgM in mice treated with TACI-Fc or BCMA-Fc fusion proteins or non-fused Fc as a control. p values refer to the comparison with the Fc-treated group. n = 7. See Materials and Methods hereinafter.

Figure 21 shows the sequence of Fc-humanAPRIL

Figure 22 shows the effect of Fc-humanAPRIL and soluble humanAGP3/BlyS/Tall-1 on proliferation of primary murine B cells. Purified murine spleen B cells were cultured in presence of various amounts of human Fc-APRIL and untagged AGP3 plus 2 µg/ml of anti-IgM . Data show incorporation of 3H thymidine as cpm, and represent mean of triplicate wells.

Figure 23 shows that hBCMA-Fc and hTACI-Fc inhibits Flag-mAPRIL mediated mouse B cell proliferation. Purified murine spleen B cells were cultured with the indicated amounts of soluble BCMA-Fc and TACI-Fc in presence of 10 ng/ml of Flag-mAPRIL and 2 µg/ml of an anti-IgM for 72 hr. Incorporation of 3H thymidine is indicated as cpm. Data shown represent mean of triplicate wells.

Figure 24 shows that administration of hBCMA-Fc (15 mg/kg intraperitoneally (ip_ on day 0, 3, and 6) reduces mouse peripheral blood B cell levels measured at day seven. Normal mice (n=7) were treated with

human BCMA-Fc and nonfused Fc as a control in day 0,3, and 6 (15 mg/kg). Peripheral blood B cell levels were measured on day 7.

Figure 25 shows that administration of hBCMA-Fc (15 mg/kg ip on day 0, 3, and 6) reduces mouse spleen B cell levels measured at day seven.

5 Figure 26 shows that Flag-mAPRIL and hAGP3 mediated IgA production is inhibited by hBCMA-Fc and hTACI-Fc *in vitro*. Purified murine spleen B cells were cultured with LPS (100 ng/ml),AGP3 (10 ng/ml),Flag-APRIL (10 ng/ml) or plus BCMA-Fc (100 ng/ml) and TACI-Fc (100 ng/ml) for 12 days. Culture supernatants were collected on day 7 and day 12 for
10 detecting IgA level.

Figure 27 shows that Flag-mAPRIL and hAGP3 mediated IgG production is inhibited by hBCMA-Fc and hTACI-Fc *in vitro*.

Figure 28 shows reduced total IgE and IgA levels in normal mice treated with mBCMA-Fc and truncated hTACI-Fc (5 mg/kg ip day 0, 3, and 6).

15 Normal mice (n=7) were treated with human BCMA-Fc ,truncated TACI-Fc and nonfused Fc as a control in day 0, 3, and 6 (5 mg/kg). Immunoglobulin levels in serum were measured on day 7.

Figure 29 shows that treatment with hBCMA-Fc or truncated-hTACI-Fc reduces anti-Pneumovacs specific IgM levels in normal mice.

20 Normal mice (n=7) were treated with human BCMA-Fc, truncated TACI-Fc and nonfused Fc as a control in daily doses of 0.5mg/kg to 15 mg/kg for 7 days. At preimmunization anti-KLH and anti-Pneumovax were undetectable. Antibodies were measured on day 7 and day 14.

Figure 30 shows that the anti-mAPRIL specific monoclonal
25 antibody #c19 inhibits Flag-mAPRIL mediated mouse B cell proliferation. Purified murine spleen B cells were cultured in presence 10 ng/ml mFlag-APRIL plus 2 ug/ml of anti-IgM . anti-Flag-APRIL monoclonal antibody c-19 and rat IgG control were added into culture in same time. Data show

incorporation of ³H thymidine as cpm, and represent mean of triplicate wells.

Figure 31 shows that treatment with the anti-mAPRIL specific monoclonal antibody #c19 inhibits generation of anti-Pneumovacs specific antibodies *in vivo*.

Figure 32 shows that treatment with hBCMA-Fc increases survival in the NZB/NZWF1 mouse model of SLE.

Figure 33 shows that treatment with hBCMA-Fc reduces the incidence of proteinuria in the NZB/NZWF1 mouse model of SLE.

Figure 34 shows that treatment with hBCMA-Fc decreases anti-dsDNA specific antibody levels in the NZB/NZWF1 mouse model of SLE.

Figure 35 shows that treatment with hBCMA-Fc decreases peripheral blood % B cells in the NZB/NZWF1 mouse model of SLE.

Fig 36 shows that APRIL binds to a number of tumor cell lines. APRIL binding to human tumor cell lines were determined by incubating cell with 1ug/ml Fc-APRIL or Flag-APRIL following FITC labeled secondary antibody staining and FACS analysis.

Figure 37 shows that APRIL stimulates U266-B1 cell growth and that this can be inhibited by BCMA-Fc or TACI-Fc. U266 cells were cultured in presence 10ng/ml human Fc-APRIL or plus 50ng/ml soluble BCMA-Fc, Truncated TACI-Fc for 48 hr. Incorporation of ³H thymidine is indicated as cpm. Data shown represent mean of triplicate wells.

Figure 38 shows that APRIL stimulates mouse B lymphoma A20 cell growth and that this can be inhibited by BCMA-Fc or TACI-Fc. A20 mouse B cell lymphoma were cultured in presence 50ng/ml mouse Flag-APRIL, human Flag-AGP3 or plus 100 ng/ml soluble BCMA-Fc for 48 hr. Incorporation of ³H thymidine is indicated as cpm. Data shown represent mean of triplicate wells.

Figure 39 shows that treatment with BCMA-Fc reduces A20 B lymphoma tumor cell growth in Balb/c mice. A20 (0.2 million) cells were implanted, id, on day 0. Treatments with PBS, CHO-Fc, mBCMA-Fc, hBCMA-Fc, mTACI-Fc, hTACI-Fc were given (10 mg/kg, ip) on days 0, 7, 10, 13, 16, 19, 22. Tumor measurements were made twice per week. Mice were sacrificed on days 27-31, tumors were snap frozen for RNA isolation, blood was collected and serum samples were frozen.

Figure 40 shows that treatment with hBCMA-Fc reduces human colon carcinoma cell line HT29 tumor volume growth in mice. 2×10^6 cells plus 50% matrigel injected subcutaneously into athymic nude mice. Rx: human BCMA-Fc at 2, 5, and 15 mg/kg Q2D, starting at day 0 (n=10/group). Control 1: CHO-Fc at 15 mg/kg Q2D. Control 2: 0.2 ml of PBS Q2D IP. Tumor volume: 3/week, from day 7. Tumor weight at end of study. Body weight 2/week.

Figure 41 shows that treatment with mBCMA-Fc reduces human colon carcinoma cell line HT29 tumor volume growth in mice. 2×10^6 cells plus 50% matrigel injected subcutaneously into athymic nude mice. Rx: mouse BCMA-Fc at 2, 5, and 15 mg/kg Q2D, starting at day 0 (n=10/group). Control 1: CHO-Fc at 15 mg/kg Q2D. Control 2: 0.2 ml of PBS Q2D IP. Tumor volume: 3/week, from day 7. Tumor weight at end of study. Body weight 2/week.

Detailed Description of the Invention

Definition of Terms

The terms used throughout this specification are defined as follows, unless otherwise limited in specific instances.

The term "comprising" means that a compound may include additional amino acids on either or both of the N- or C- termini of the given sequence. Of course, these additional amino acids should not significantly interfere with the activity of the compound.

“AGP-3 activity” refers to modulation of cell growth, survival, or activation resulting from binding by natural human AGP-3 to TACI or BCMA, particularly in B cells. Conversely, “AGP-3 antagonist activity” refers to activity in opposition to AGP-3 activity, as would result, for example, by inhibition of binding of AGP-3 to TACI or BCMA. Such activity can be determined, for example, by such assays as described in “Biological activity of AGP-3” in the Materials & Methods of PCT/US00/03653, which is hereby incorporated by reference. Additional assays by which AGP-3 activity may be identified appear in the references WO 98/18921 (May 7, 1998); WO 98/27114 (June 25, 1998); EP 869 180 (October 7, 1998); WO 98/55620 and WO 98/55621 (December 10, 1998); WO 99/11791 (March 11, 1999); WO99/12964 (March 18, 1999); and Gross *et al.* (2000), *Nature* 404: 995-9. Any of the assays described therein may be modified as needed by methods known to persons having ordinary skill in the art.

“APRIL activity” refers to modulation of cell growth, survival, or activation resulting from binding of natural human APRIL to TACI or BCMA, particularly in T cells. Conversely, “APRIL antagonist activity” refers to activity in opposition to APRIL activity, as would result, for example, by inhibition of binding of APRIL to TACI or BCMA. Such activity can be determined, for example, by such assays as described in the Materials & Methods hereinafter. Additional assays by which APRIL activity may be identified appear in the references WO 99/00518 (June 26, 1997); WO 99/11791 (Sept. 5, 1997); WO 99/12965 (Sept. 12, 1997); EP 911 633 (Oct. 8, 1997); EP 919 620 (Nov. 26, 1997); WO 99/28462 (Dec. 3, 1997); WO 99/33980 (Dec. 30, 1997); WO 99/35170 (Jan. 5, 1998); and Hahne *et al.* (1998), *J. Exp. Med.* 188: 1185-90. Any of the assays described therein and herein may be modified as needed by methods known to persons having ordinary skill in the art.

“BCMA activity” refers to modulation of cell growth, survival, or activation resulting from binding by natural human APRIL or natural human AGP-3 to BCMA. Conversely, “BCMA antagonist activity” refers to activity in opposition to BCMA activity, as would result, for example, by inhibition of binding of AGP-3 or APRIL to BCMA. Such activity can be determined, for example, by such assays as described in the Materials & Methods hereinafter. Additional assays by which BCMA activity may be identified appear in the references WO 99/00518 (June 26, 1997); WO 99/11791 (Sept. 5, 1997); WO 99/12965 (Sept. 12, 1997); EP 911 633 (Oct. 8, 1997); EP 919 620 (Nov. 26, 1997); WO 99/28462 (Dec. 3, 1997); WO 99/33980 (Dec. 30, 1997); WO 99/35170 (Jan. 5, 1998); Hahne *et al.* (1998), *J. Exp. Med.* 188: 1185-90; WO 98/18921 (May 7, 1998); WO 98/27114 (June 25, 1998); EP 869 180 (October 7, 1998); WO 98/55620 and WO 98/55621 (December 10, 1998); WO 99/11791 (March 11, 1999); WO99/12964 (March 18, 1999); and Gross *et al.* (2000), *Nature* 404: 995-9. Any of the assays described therein and herein may be modified as needed by methods known to persons having ordinary skill in the art.

“TACI activity” refers to modulation of cell growth, survival, or activation resulting from binding by natural human AGP-3 or natural human APRIL to TACI. Conversely, “TACI antagonist activity” refers to activity in opposition to TACI activity, as would result, for example, by inhibition of binding of AGP-3 or APRIL to TACI. Such activity can be determined, for example, by such assays as described in the Materials & Methods of PCT/US00/03653, WO 98/18921 (May 7, 1998), WO 98/27114 (June 25, 1998), EP 869 180 (October 7, 1998), WO 98/55620 and WO 98/55621 (December 10, 1998), WO 99/11791 (March 11, 1999), WO99/12964 (March 18, 1999), WO 98/39361 (September 11, 1998), von Bulow & Bram (1997), *Science*, 278:138-140, and Gross *et al.* (2000), *Nature*

404: 995-9. Any of the assays described therein may be modified as needed by methods known to persons having ordinary skill in the art.

The term "specific binding partner" refers to any molecule that preferentially binds to a protein of interest, regardless of the antagonistic
5 or agonistic activity of the molecule toward the protein of interest. Exemplary specific binding partners include antibodies, solubilized receptors, peptides, modified peptides as described hereinafter, and the like.

The term "vehicle" refers to a molecule that prevents degradation
10 and/or increases half-life, reduces toxicity, reduces immunogenicity, or increases biological activity of a therapeutic protein. Exemplary vehicles include an Fc domain (which is preferred) as well as a linear polymer (e.g., polyethylene glycol (PEG), polylysine, dextran, etc.); a branched-chain polymer (see, for example, U.S. Patent No. 4,289,872 to Denkenwalter et
15 al., issued September 15, 1981; 5,229,490 to Tam, issued July 20, 1993; WO 93/21259 by Frechet et al., published 28 October 1993); a lipid; a cholesterol group (such as a steroid); a carbohydrate or oligosaccharide; or any natural or synthetic protein, polypeptide or peptide that binds to a salvage receptor. Vehicles are further described hereinafter.

20 The term "native Fc" refers to molecule or sequence comprising the sequence of a non-antigen-binding fragment resulting from digestion of whole antibody, whether in monomeric or multimeric form. The original immunoglobulin source of the native Fc is preferably of human origin and may be any of the immunoglobulins, although IgG1 and IgG2 are
25 preferred. Native Fc's are made up of monomeric polypeptides that may be linked into dimeric or multimeric forms by covalent (i.e., disulfide bonds) and non-covalent association. The number of intermolecular disulfide bonds between monomeric subunits of native Fc molecules ranges from 1 to 4 depending on class (e.g., IgG, IgA, IgE) or subclass (e.g.,

IgG1, IgG2, IgG3, IgA1, IgGA2). One example of a native Fc is a disulfide-bonded dimer resulting from papain digestion of an IgG (see Ellison *et al.* (1982), Nucleic Acids Res. 10: 4071-9). The term "native Fc" as used herein is generic to the monomeric, dimeric, and multimeric forms.

5 The term "Fc variant" refers to a molecule or sequence that is modified from a native Fc but still comprises a binding site for the salvage receptor, FcRn. International applications WO 97/34631 (published 25 September 1997) and WO 96/32478 describe exemplary Fc variants, as well as interaction with the salvage receptor, and are hereby incorporated
10 by reference. Thus, the term "Fc variant" comprises a molecule or sequence that is humanized from a non-human native Fc. Furthermore, a native Fc comprises sites that may be removed because they provide structural features or biological activity that are not required for the fusion molecules of the present invention. Thus, the term "Fc variant" comprises
15 a molecule or sequence that lacks one or more native Fc sites or residues that affect or are involved in (1) disulfide bond formation, (2) incompatibility with a selected host cell (3) N-terminal heterogeneity upon expression in a selected host cell, (4) glycosylation, (5) interaction with complement, (6) binding to an Fc receptor other than a salvage receptor, or
20 (7) antibody-dependent cellular cytotoxicity (ADCC). Fc variants are described in further detail in WO 00/24782, published May 4, 2000, which is hereby incorporated by reference in its entirety.

 The term "Fc domain" encompasses native Fc and Fc variant molecules and sequences as defined above. As with Fc variants and native
25 Fc's, the term "Fc domain" includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

 The term "multimer" as applied to Fc domains or molecules comprising Fc domains refers to molecules having two or more

polypeptide chains associated covalently, noncovalently, or by both covalent and non-covalent interactions. IgG molecules typically form dimers; IgM, pentamers; IgD, dimers; and IgA, monomers, dimers, trimers, or tetramers. Multimers may be formed by exploiting the
5 sequence and resulting activity of the native Ig source of the Fc or by derivatizing (as defined below) such a native Fc.

The term "dimer" as applied to Fc domains or molecules comprising Fc domains refers to molecules having two polypeptide chains associated covalently or non-covalently.

10 The terms "derivatizing" and "derivative" or "derivatized" comprise processes and resulting compounds respectively in which (1) the compound has a cyclic portion; for example, cross-linking between cysteinyl residues within the compound; (2) the compound is cross-linked or has a cross-linking site; for example, the compound has a cysteinyl
15 residue and thus forms cross-linked dimers in culture or in vivo; (3) one or more peptidyl linkage is replaced by a non-peptidyl linkage; (4) the N-terminus is replaced by $-NRR^1$, $NRC(O)R^1$, $-NRC(O)OR^1$, $-NRS(O)_2R^1$, $-NHC(O)NHR$, a succinimide group, or substituted or unsubstituted benzyloxycarbonyl-NH-, wherein R and R^1 and the ring substituents are
20 as defined hereinafter; (5) the C-terminus is replaced by $-C(O)R^2$ or $-NR^3R^4$ wherein R^2 , R^3 and R^4 are as defined hereinafter; and (6) compounds in which individual amino acid moieties are modified through treatment with agents capable of reacting with selected side chains or terminal residues. Derivatives are further described hereinafter.

25 The term "peptide" refers to molecules of 2 to 40 amino acids, with molecules of 3 to 20 amino acids preferred and those of 6 to 15 amino acids most preferred. Exemplary peptides may be randomly generated by any of the methods cited above, carried in a peptide library (e.g., a phage display library), or derived by digestion of proteins.

The term "randomized" as used to refer to peptide sequences refers to fully random sequences (e.g., selected by phage display methods) and sequences in which one or more residues of a naturally occurring molecule is replaced by an amino acid residue not appearing in that position in the naturally occurring molecule. Exemplary methods for identifying peptide sequences include phage display, *E. coli* display, ribosome display, yeast-based screening, RNA-peptide screening, chemical screening, rational design, protein structural analysis, and the like. Randomized peptides and methods of generating them appear in WO 00/24782, published May 4, 2000, which is hereby incorporated by reference in its entirety.

The term "pharmacologically active" means that a substance so described is determined to have activity that affects a medical parameter (e.g., T cell proliferation) or disease state (e.g., cancer, autoimmune disorders). Thus, pharmacologically active compounds comprise agonistic or mimetic and antagonistic compounds as defined below.

The terms "-mimetic" and "agonist" refer to a molecule having biological activity comparable to a protein (e.g., APRIL, AGP-3) that interacts with a protein of interest. These terms further include molecules that indirectly mimic the activity of a protein of interest, such as by potentiating the effects of the natural ligand of the protein of interest.

The terms "antagonist" or "inhibitor" refer to a molecule that blocks or in some way interferes with the biological activity of the associated protein of interest, or has biological activity comparable to a known antagonist or inhibitor of the associated protein of interest.

Additionally, physiologically acceptable salts of the compounds of this invention are also encompassed herein. By "physiologically acceptable salts" is meant any salts that are known or later discovered to be pharmaceutically acceptable. Some specific examples are: acetate;

trifluoroacetate; hydrohalides, such as hydrochloride and hydrobromide; sulfate; citrate; tartrate; glycolate; and oxalate.

Methods of Treatment

The present invention concerns a method of inhibiting T cell proliferation in a mammal, which comprises administering a therapeutic agent comprising:

- a. a specific binding partner for TACI, wherein the specific binding partner has TACI antagonist activity;
- b. a specific binding partner for BCMA, wherein the specific binding partner has BCMA antagonist activity;
- c. both a and b; or
- d. a specific binding partner for TACI and BCMA, wherein the specific binding partner has TACI antagonist activity, BCMA antagonist activity or both.

The present invention also concerns a method of inhibiting APRIL activity in a mammal, which comprises administering a therapeutic agent comprising a through d above.

The invention also concerns a method of inhibiting TACI activity, BCMA activity, or both in a mammal, which comprises administering a specific binding partner for APRIL. This method may further comprise administering a specific binding partner for AGP-3.

Some indications benefit from an increase in the immune response. Accordingly, the invention further relates to a method of increasing T cell proliferation in a mammal, which comprises administering a therapeutic agent comprising:

- a. a specific binding partner for TACI, wherein the specific binding partner has TACI agonist activity;
- b. a specific binding partner for BCMA, wherein the specific binding partner has BCMA agonist activity;

- c. both a and b; or
- d. a specific binding partner for TACI and BCMA, wherein the specific binding partner has TACI agonist activity, BCMA agonist activity or both.

5 The invention also concerns a method of increasing APRIL activity in a mammal, which comprises administering a therapeutic agent comprising a through d above.

 The inventors contemplate carrying out the foregoing methods of treatment with any of several different types of molecules, including small
10 molecules, antibodies, and engineered peptides and fusion molecules described hereinafter. These molecules may also be used in assays to identify cells and tissues that express AGP-3, TACI, APRIL, or BCMA. The invention further concerns nucleic acids, vectors, and host cells useful in preparing such molecules.

15 The invention further concerns methods of identifying compounds that are useful in the aforementioned methods of use. Such compounds include nucleic acids, peptides, proteins, carbohydrates, lipids or small molecular weight organic molecules and may act either as agonists or antagonists of BCMA, TACI, AGP-3 or APRIL-protein activity.

20 AGP-3, APRIL, BCMA, and TACI are believed to play a role in regulation of immune function. Accordingly, these molecules, their soluble forms, and agonists and antagonists thereof may be useful for the diagnosis and/or treatment of inflammation and immune function diseases. Indications for antagonists include, but are not limited to the
25 following:

- infections such as bacterial, fungal, protozoan and viral infections, especially HIV-1 or HIV-2;
- diarrhorea;
- psoriasis;

- inflammation;
- allergies;
- atopic dermatitis;
- respiratory allergic diseases such as asthma, allergic rhinitis,
- 5 hypersensitivity lung disease, hypersensitivity pneumonitis, eosinophilic pneumonia (e.g. Loeffler's syndrome, chronic eosinophilic pneumonia, interstitial lung disease (ILD), such as idiopathic pulmonary fibrosis or ILD associated with rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis,
- 10 systemic sclerosis, Sjogren's syndrome, polymyositis or dermatomyositis);
- systemic anaphylaxis or hypersensitivity responses;
- drug allergy;
- insect sting allergy;
- 15 • inflammatory bowel disease, such as Crohn's disease and ulcerative colitis;
- spondyloarthropathy;
- scleroderma;
- psoriasis;
- 20 • inflammatory dermatosis such as dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria, vasculitis (e.g. necrotizing, cutaneous and hypersensitivity vasculitis), eosinophilic myositis and eosinophilic fasciitis;
- autoimmune diseases such as rheumatoid arthritis, psoriatic
- 25 arthritis, multiple sclerosis, systemic lupus erythematosus, myasthenia gravis, juvenile onset diabetes, glomerulonephritis, autoimmune thyroiditis and Behcet's disease;
- graft rejection, including allograft rejection or graft-versus-host disease;

- cancers with leukocyte infiltration of the skin or organs;
- reperfusion injury;
- atherosclerosis;
- certain haematologic malignancies;
- 5 • shock, including septic shock and endotoxic shock.

Agonists can be used for treating:

- immunosuppression e.g. in AIDS patients or individuals undergoing radiation therapy, chemotherapy, therapy for autoimmune disease or other drug therapy, and
- 10 immunosuppression due congenital deficiency in receptor function or other causes; and
- infectious diseases such as parasitic diseases, including helminth infections, such as nematodes (round worms).

Compositions of matter

15 Any number of molecules may serve as specific binding partners within the present invention. Of particular interest are antibodies, peptides, and Fc-peptide fusion molecules.

Antibodies.

20 The invention also provides for an antibody or antigen binding domain thereof, or a fragment, variant, or derivative thereof, which binds to an epitope on any of the target molecules (APRIL, AGP-3, TACI, or BCMA) and has partial or complete agonist or antagonist activity. Preferably, the target molecule is mammalian, more preferably human, and may be in soluble or cell surface associated forms, or fragments,

25 derivatives and variants thereof.

A number of methods for antibody generation are known in the art. All such methods are useful in generating molecules useful in accordance with the present invention. Conventionally, an antibody may be prepared by immunizing an animal with the target molecule (e.g., murine or human

BCMA or TACI) or with an immunogenic fragment, derivative or variant thereof. In addition, an animal may be immunized with cells transfected with a vector containing a nucleic acid molecule encoding the target molecule such that the target molecule is expressed and associated with the surface of the transfected cells. Alternatively, specific binding partners that are antibodies may be obtained by screening a library comprising antibody or antigen binding domain sequences for binding to the target molecule. Such a library is conveniently prepared in bacteriophage as protein or peptide fusions to a bacteriophage coat protein which are expressed on the surface of assembled phage particles and the encoding DNA sequences contained within the phage particles (so-called "phage display library"). In one example, a phage display library contains DNA sequences encoding human antibodies, such as variable light and heavy chains. Sequences binding to the target molecule may be further evolved by multiple rounds of mutagenesis and screening.

Specific binding partners that are antibodies or antigen binding domains may be tetrameric glycoproteins similar to native antibodies, or they may be single chain antibodies; for example, Fv, Fab, Fab' or F(ab)' fragments, bispecific antibodies, heteroantibodies, or other fragments, variants, or derivatives thereof, which are capable of binding the target molecule and partially or completely neutralize the target molecule activity. Antibodies or antigen binding domains may be produced in hybridoma cell lines (antibody-producing cells such as spleen cells fused to mouse myeloma cells, for example) or may be produced in heterologous cell lines transfected with nucleic acid molecules encoding said antibody or antigen binding domain.

Antibodies of the invention include polyclonal monospecific polyclonal, monoclonal, recombinant, chimeric, humanized, fully human, single chain and/or bispecific antibodies. Antibody fragments include

those portions of an antibody that bind to an epitope on a target molecule. Examples of such fragments include Fab F(ab'), F(ab)'₂, Fv, and sFv fragments. The antibodies may be generated by enzymatic cleavage of full-length antibodies or by recombinant DNA techniques, such as
5 expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen. An antigen is a molecule or a portion of a molecule capable of being
10 bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen can have one or more epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of
15 other antibodies which can be evoked by other antigens.

Polyclonal antibodies directed toward a target molecule generally are raised in animals (e.g., rabbits or mice) by multiple subcutaneous or intraperitoneal injections of the target molecule and an adjuvant. In accordance with the invention, it may be useful to conjugate the target
20 molecule, or a variant, fragment, or derivative thereof to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet heocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance the immune response. After immunization, the animals are bled
25 and the serum is assayed for anti-target antibody titer.

Monoclonal antibodies (mAbs) contain a substantially homogeneous population of antibodies specific to antigens, which population contains substantially similar epitope binding sites. Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE,

IgA, IgD and any subclass thereof. A hybridoma producing a monoclonal antibody of the present invention may be cultivated in vitro, in situ, or in vivo. Production of high titers in vivo or in situ is a preferred method of production.

5 Monoclonal antibodies directed toward the target molecule are produced using any method which provides for the production of antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include hybridoma methods of Kohler et al., Nature 256, 495-497 (1975), and the human B-cell
10 hybridoma method, Kozbor, J. Immunol. 133, 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory (1988); the contents of which references are incorporated entirely herein by reference.

15 Preferred specific binding partners include monoclonal antibodies which will inhibit partially or completely the binding of the human target molecule to its cognate ligand or receptor or an antibody having substantially the same specific binding characteristics, as well as fragments and regions thereof. Preferred methods for determining
20 monoclonal antibody specificity and affinity by competitive inhibition can be found in Harlow et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Colligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, Meth. Enzymol., 92:589-
25 601 (1983). Each of these references is incorporated herein by reference in its entirety.

 Also provided by the invention are hybridoma cell lines which produce monoclonal antibodies reactive with target polypeptides.

Chimeric antibodies are molecules in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Chimeric antibodies are primarily used
5 to reduce immunogenicity in application and to increase yields in production, for example, where murine monoclonal antibodies have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric monoclonal antibodies are used.

Chimeric antibodies and methods for their production are known
10 in the art. Cabilly et al., Proc. Natl. Acad. Sci. USA, 81:3273-3277 (1984); Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984); Boulianne et al., Nature, 312:643-646 (1984); Neuberger et al., Nature, 314:268-270 (1985); Liu et al., Proc. Natl. Acad. Sci. USA, 84:3439-3443 (1987); and Harlow and Lane Antibodies: A Laboratory Manual, Cold Spring Harbor
15 Laboratory (1988). These references are incorporated herein by reference in their entirety.

A chimeric monoclonal antibody of the invention may be used as a therapeutic agent. In such a chimeric antibody, a portion of the heavy and/or light chain is identical with or homologous to corresponding
20 sequence in antibodies derived from a particular species or belonging to one particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they
25 exhibit the desired biological activity (see U.S. Patent No. 4,816,567; Morrison et al., Proc. Natl. Acad. Sci., 81, 6851-6855 (1985)).

As used herein, the term "chimeric antibody" includes monovalent, divalent or polyvalent immunoglobulins. A monovalent chimeric antibody is a dimer (HL) formed by a chimeric H chain associated through

disulfide bridges with a chimeric L chain. A divalent chimeric antibody is tetramer (H_2L_2) formed by two HL dimers associated through at least one disulfide bridge. A polyvalent chimeric antibody can also be produced, for example, by employing a C_H region that aggregates (e.g., from an IgM
5 H chain, or μ chain).

Murine and chimeric antibodies, fragments and regions of the present invention may comprise individual heavy (H) and/or light (L) immunoglobulin chains. A chimeric H chain comprises an antigen binding region derived from the H chain of a non-human antibody
10 specific for the target molecule, which is linked to at least a portion of a human H chain C region (C_H), such as CH_1 or CH_2 .

A chimeric L chain according to the present invention comprises an antigen binding region derived from the L chain of a non-human antibody specific for the target molecule, linked to at least a portion of a human L
15 chain C region (C_L).

Specific binding partners, such as antibodies, fragments, or derivatives, having chimeric H chains and L chains of the same or different variable region binding specificity, can also be prepared by appropriate association of the individual polypeptide chains, according to
20 known method steps, e.g., according to Ausubel *et al.*, eds. Current Protocols in Molecular Biology, Wiley Interscience, N.Y. (1993), and Harlow *et al.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1988). The contents of these references are incorporated entirely herein by reference. With this
25 approach, hosts expressing chimeric H chains (or their derivatives) are separately cultured from hosts expressing chimeric L chains (or their derivatives), and the immunoglobulin chains are separately recovered and then associated. Alternatively, the hosts can be co-cultured and the chains

allowed to associate spontaneously in the culture medium, followed by recovery of the assembled immunoglobulin, fragment or derivative.

As an example, the antigen binding region of the specific binding partner (such as a chimeric antibody) of the present invention is preferably
5 derived from a non-human antibody specific for the human analog of the target molecule. Preferred sources for the DNA encoding such a non-human antibody include cell lines which produce antibodies, such as hybrid cell lines commonly known as hybridomas.

The invention also provides for fragments, variants and derivatives,
10 and fusions of anti-target antibodies, wherein the terms "fragments", "variants", "derivatives" and "fusions" are defined herein. The invention encompasses fragments, variants, derivatives, and fusions of anti-target antibodies which are functionally similar to the unmodified antibody, that is, they retain at least one of the activities of the unmodified antibody. In
15 addition to the modifications set forth above, also included is the addition of genetic sequences coding for cytotoxic proteins such as plant and bacterial toxins. The fragments, variants, derivatives and fusions of the antibodies can be produced from any of the hosts of this invention.

Suitable fragments include, for example, Fab, Fab', F(ab')₂, Fv and
20 scFv. These fragments lack the Fc fragment of an intact antibody, clear more rapidly from the circulation, and can have less non-specific tissue binding than an intact antibody. See Wahl *et al.*, *J. Nucl. Med.*, 24:316-325 (1983). These fragments are produced from intact antibodies using methods well known in the art, for example by proteolytic cleavage with
25 enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). The identification of these antigen binding regions and/or epitopes recognized by monoclonal antibodies of the present invention provides the information necessary to generate additional monoclonal antibodies with similar binding characteristics and

therapeutic or diagnostic utility that parallel the embodiments of this invention.

5 Variants of specific binding partners are also provided. In one embodiment, variants of antibodies and antigen binding domains comprise changes in light and/or heavy chain amino acid sequences that are naturally occurring or are introduced by in vitro engineering of native sequences using recombinant DNA techniques. Naturally occurring variants include "somatic" variants which are generated in vivo in the corresponding germ line nucleotide sequences during the generation of an
10 antibody response to a foreign antigen.

Variants of antibodies and antigen binding domains are also prepared by mutagenesis techniques known in the art. In one example, amino acid changes may be introduced at random throughout an antibody coding region and the resulting variants may be screened for a desired
15 activity, such as binding affinity for the target molecule. Alternatively, amino acid changes may be introduced in selected regions of an antibody, such as in the light and/or heavy chain CDRs, and framework regions, and the resulting antibodies may be screened for binding to the target molecule or some other activity. Amino acid changes encompass one or
20 more amino acid substitutions in a CDR, ranging from a single amino acid difference to the introduction of all possible permutations of amino acids within a given CDR, such as CDR3. In another method, the contribution of each residue within a CDR to target binding may be assessed by substituting at least one residue within the CDR with alanine (Lewis et al.
25 (1995), Mol. Immunol. 32: 1065-72). Residues which are not optimal for binding to the target molecule may then be changed in order to determine a more optimum sequence. Also encompassed are variants generated by insertion of amino acids to increase the size of a CDR, such as CDR3. For example, most light chain CDR3 sequences are nine amino acids in length.

Light chain CDR3 sequences in an antibody which are shorter than nine residues may be optimized for binding to the target molecule by insertion of appropriate amino acids to increase the length of the CDR.

In one embodiment, antibody or antigen binding domain variants
5 comprise one or more amino acid changes in one or more of the heavy or light chain CDR1, CDR2 or CDR3 and optionally one or more of the heavy or light chain framework regions FR1, FR2 or FR3. Amino acid changes comprise substitutions, deletions and/or insertions of amino acid residues.

10 Variants may also be prepared by "chain shuffling" of either light or heavy chains. Marks *et al.* (1992), *Biotechnology* 10: 779-83. Typically, a single light (or heavy) chain is combined with a library having a repertoire of heavy (or light) chains and the resulting population is screened for a desired activity, such as binding to the target molecule. This technique
15 permits screening of a greater sample of different heavy (or light) chains in combination with a single light (or heavy) chain than is possible with libraries comprising repertoires of both heavy and light chains.

The specific binding partners of the invention can be bispecific. Bispecific specific binding partners of this invention can be of several
20 configurations. For example, bispecific antibodies resemble single antibodies (or antibody fragments) but have two different antigen binding sites (variable regions). Bispecific antibodies can be produced by chemical techniques (see *e.g.*, Kranz *et al.*, *Proc. Natl. Acad. Sci. USA*, 78:5807 (1981)), by "polydoma" techniques (see U.S. Pat. No. 4,474,893 to Reading)
25 or by recombinant DNA techniques. For example, a bispecific antibody in accordance with this invention may bind to APRIL and AGP-3. As another example, a bispecific antibody may bind to TACI and BCMA.

The specific binding partners of the invention may also be heteroantibodies. Heteroantibodies are two or more antibodies, or

antibody binding fragments (Fab) linked together, each antibody or fragment having a different specificity.

The invention also relates to "humanized" antibodies. Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into a human antibody from a source which is non-human. In general, non-human residues will be present in CDRs. Humanization can be performed following methods known in the art (Jones *et al.*, *Nature* 321, 522-525 (1986); Riechmann *et al.*, *Nature*, 332, 323-327 (1988); Verhoeyen *et al.*, *Science* 239, 1534-1536 (1988)), by substituting rodent complementarily-determining regions (CDRs) for the corresponding regions of a human antibody.

The specific binding partners of the invention, including chimeric, CDR-grafted, and humanized antibodies can be produced by recombinant methods known in the art. Nucleic acids encoding the antibodies are introduced into host cells and expressed using materials and procedures described herein and known in the art. In a preferred embodiment, the antibodies are produced in mammalian host cells, such as CHO cells. Fully human antibodies may be produced by expression of recombinant DNA transfected into host cells or by expression in hybridoma cells as described above.

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules which bypass the generation of monoclonal antibodies are encompassed within the practice of this invention. To do so, antibody-specific messenger RNA molecules are extracted from immune system cells taken from an immunized animal, and transcribed into complementary DNA (cDNA). The cDNA is then cloned into a bacterial expression system. One example of such a technique suitable for the practice of this invention uses a bacteriophage

lambda vector system having a leader sequence that causes the expressed Fab protein to migrate to the periplasmic space (between the bacterial cell membrane and the cell wall) or to be secreted. One can rapidly generate and screen great numbers of functional Fab fragments for those which
5 bind the antigen. Such target molecule specific binding partners (Fab fragments with specificity for the target molecule) are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

Also within the scope of the invention are techniques developed for
10 the production of chimeric antibodies by splicing the genes from a mouse antibody molecule of appropriate antigen-specificity together with genes from a human antibody molecule of appropriate biological activity, such as the ability to activate human complement and mediate ADCC. (Morrison *et al.*, *Proc. Natl. Acad. Sci.*, 81:6851 (1984); Neuberger *et al.*,
15 *Nature*, 312:604 (1984)). One example is the replacement of a Fc region with that of a different isotype. Specific binding partners such as antibodies produced by this technique are within the scope of the invention.

In a preferred embodiment of the invention, the antibodies are fully
20 human antibodies. Thus encompassed by the invention are antibodies that bind target molecules and are encoded by nucleic acid sequences which are naturally occurring somatic variants of human germline immunoglobulin nucleic acid sequence, and fragments, synthetic variants, derivatives and fusions thereof. Such antibodies may be produced by any
25 method known in the art. Exemplary methods include immunization with a target antigen (any target polypeptide capable of eliciting an immune response, and optionally conjugated to a carrier) of transgenic animals (*e.g.*, mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. See, for

example, Jakobovits *et al.*, Proc. Natl. Acad. Sci., 90, 2551-2555 (1993); Jakobovits *et al.*, Nature, 362, 255-258 (1993); Bruggermann *et al.*, Year in Immunol., 7, 33 (1993).

Alternatively, human antibodies may be generated through the *in vitro* screening of phage display antibody libraries. See Hoogenboom *et al.*, J. Mol. Biol., 227, 381 (1991); Marks *et al.*, J. Mol. Biol., 222, 581 (1991), incorporated herein by reference. Various antibody-containing phage display libraries have been described and may be readily prepared by one skilled in the art. Libraries may contain a diversity of human antibody sequences, such as human Fab, Fv, and scFv fragments, that may be screened against an appropriate target. As described further below, phage display libraries may comprise peptides or proteins other than antibodies which may be screened to identify specific binding partners of the target molecule.

An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the same species and genetic type (e.g., mouse strain) as the source of the monoclonal antibody with the monoclonal antibody to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody). See, for example, U.S. Pat. No. 4,699,880, which is herein entirely incorporated by reference. The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may be epitopically identical to the original monoclonal antibody which induced the anti-Id. Thus, by using

antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

Peptides and Peptide fusion molecules.

The patent application WO 00/24782, published May 4, 2000,
5 mentioned previously herein describes in detail various peptide generation techniques. That patent application further describes various derivatives and fusion molecules.

In particular, a peptide used as a specific binding partner may be comprised within a molecule of the formula



wherein:

F^1 is a vehicle;

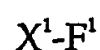
X^1 and X^2 are each independently selected from $-(L^1)_c-P^1$, $-(L^1)_c-P^1-(L^2)_d-P^2$, $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$, and $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$

15 P^1 , P^2 , P^3 , and P^4 are each independently peptide sequences, wherein at least one is a specific binding partner;

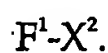
L^1 , L^2 , L^3 , and L^4 are each independently linkers; and

a , b , c , d , e , and f are each independently 0 or 1, provided that at least one of a and b is 1.

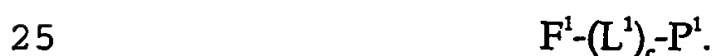
20 Preferably, such a molecule comprises a structure of the formulae



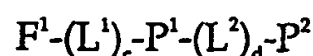
or



A more preferred molecule comprises a structure of the formula



or a structure of the formula



wherein P^1 and/or P^2 is a specific binding partner for TACI or BCMA.

Such molecules facilitate modulation of both TACI and BCMA; for

example, one of P¹ and P² is a specific binding partner for TACI and the other is a specific binding partner for BCMA. Conversely, in a ligand inhibitor, one of P¹ and P² is a specific binding partner for APRIL and the other is a specific binding partner for AGP-3.

5 For all of these molecules, the preferred vehicle is an Fc domain. Among Fc domains, IgG Fc, particularly IgG1, are preferred.

The Fc domains, linkers, and processes of preparation of the foregoing molecules is described in WO 00/24782, published May 4, 2000.

Soluble receptor fragments

10 Another class of specific binding partners are soluble receptor fragments. Of particular interest are the fragments identified in the figures:

- a. the extracellular region of TACI (SEQ ID NO: 15).
- b. the extracellular region of BCMA (SEQ ID NO: 6).
- 15 c. the consensus region of TACI (SEQ ID NO: 16).
- d. the consensus region of BCMA (SEQ ID NO: 7).
- e. the TACI/BCMA extracellular consensus sequence (SEQ ID NO: 13).

These molecules have the heretofore unrecognized advantage of binding
20 both APRIL and AGP-3. Like the aforementioned peptides, these specific binding partners may also be covalently linked to a vehicle, preferably an Fc domain.

Muteins

Additional useful peptide sequences may result from conservative
25 and/or non-conservative modifications of the amino acid sequences of the aforementioned antibodies, peptides, Fc-fusion peptides, and receptor fragments.

Conservative modifications will produce molecules having functional and chemical characteristics similar to those of the molecule

from which such modifications are made. In contrast, substantial modifications in the functional and/or chemical characteristics of the molecules may be accomplished by selecting substitutions in the amino acid sequence that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the size of the molecule.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis" (see, for example, MacLennan et al., 1998, Acta Physiol. Scand. Suppl. 643:55-67; Sasaki et al., 1998, Adv. Biophys. 35:1-24, which discuss alanine scanning mutagenesis).

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the molecule sequence, or to increase or decrease the affinity of the molecules described herein. Exemplary amino acid substitutions are set forth in Table 3.

Table 3—Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser, Ala	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro, Ala	Ala
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, 1,4 Diamino-butyrac Acid, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala, Tyr	Leu
Pro (P)	Ala	Gly
Ser (S)	Thr, Ala, Cys	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

In certain embodiments, conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems.

As noted in the foregoing section "Definition of Terms," naturally occurring residues may be divided into classes based on common sidechain properties that may be useful for modifications of sequence. For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the molecule that are homologous with non-human orthologs, or into the non-homologous regions of the molecule. In addition, one may also make modifications using P or G for the purpose of influencing chain orientation.

In making such modifications, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte et al., J. Mol. Biol., 157: 105-131 (1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those which

are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. The greatest
5 local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.*, with a biological property of the protein.

The following hydrophilicity values have been assigned to amino
10 acid residues: arginine (+3.0); lysine (+3.0); aspartate ($+3.0 \pm 1$); glutamate ($+3.0 \pm 1$); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). In making changes based
15 upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are
20 also referred to as "epitopic core regions."

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth in the foregoing sequences using well known techniques. For identifying suitable areas of the molecule that may be changed without destroying activity, one skilled in the art may target
25 areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a molecule to similar molecules. With such a comparison, one can identify residues and portions of the molecules that are conserved

among similar polypeptides. It will be appreciated that changes in areas of a molecule that are not conserved relative to such similar molecules would be less likely to adversely affect the biological activity and/or structure of the molecule. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the molecule structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar molecules that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a molecule that correspond to amino acid residues that are important for activity or structure in similar molecules. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues of the molecules.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polymolecules. In view of that information, one skilled in the art may predict the alignment of amino acid residues of a molecule with respect to its three dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such data could be used to

gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from
5 such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., Curr. Op. in Biotech., 7(4):
10 422-427 (1996), Chou et al., Biochemistry, 13(2): 222-245 (1974); Chou et al., Biochemistry, 113(2): 211-222 (1974); Chou et al., Adv. Enzymol. Relat. Areas Mol. Biol., 47: 45-148 (1978); Chou et al., Ann. Rev. Biochem., 47: 251-276 and Chou et al., Biophys. J., 26: 367-384 (1979). Moreover, computer programs are currently available to assist with predicting
15 secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural data base (PDB) has provided enhanced
20 predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm et al., Nucl. Acid. Res., 27(1): 244-247 (1999). It has been suggested (Brenner et al., Curr. Op. Struct. Biol., 7(3): 369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number
25 of structures have been resolved, structural prediction will gain dramatically in accuracy.

Additional methods of predicting secondary structure include "threading" (Jones, D., Curr. Opin. Struct. Biol., 7(3): 377-87 (1997); Sippl et al., Structure, 4(1): 15-9 (1996)), "profile analysis" (Bowie et al., Science,

253: 164-170 (1991); Gribskov et al., Meth. Enzym., 183: 146-159 (1990); Gribskov et al., Proc. Nat. Acad. Sci., 84(13): 4355-8 (1987)), and "evolutionary linkage" (See Home, supra, and Brenner, supra).

Production of specific binding partners

5 When the specific binding partner to be prepared is a proteinaceous specific binding partner, such as an antibody or an antigen binding domain or an Fc-peptide fusion molecule, various biological or chemical methods for producing said partner are available.

 Biological methods are preferable for producing sufficient
10 quantities of a specific binding partner for therapeutic use. Standard recombinant DNA techniques are particularly useful for the production of antibodies and antigen binding domains of the invention. Exemplary expression vectors, host cells and methods for recovery of the expressed product are described below.

15 A nucleic acid molecule encoding an antibody or antigen binding domain is inserted into an appropriate expression vector using standard ligation techniques. The vector is typically selected to be functional in the particular host cell employed (i.e., the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of
20 the gene can occur). A nucleic acid molecule encoding an antibody may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems) and/or eukaryotic host cells. Selection of the host cell will depend in part on whether an antibody is to be post-translationally modified (e.g., glycosylated and/or phosphorylated). If so, yeast, insect, or mammalian
25 host cells are preferable. For a review of expression vectors, see Meth. Enz. v. 185, (D.V. Goeddel, ed.), Academic Press Inc., San Diego, CA (1990)

 Typically, expression vectors used in any host cells will contain one or more of the following components: a promoter, one or more enhancer

sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a leader sequence for secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these sequences is discussed in more detail below.

The vector components may be homologous (i.e., from the same species and/or strain as the host cell), heterologous (i.e., from a species other than the host cell species or strain), hybrid (i.e., a combination of different sequences from more than one source), synthetic, or native sequences which normally function to regulate immunoglobulin expression. As such, a source of vector components may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the components are functional in, and can be activated by, the host cell machinery.

An origin of replication is selected based upon the type of host cell being used for expression. For example, the origin of replication from the plasmid pBR322 (Product No. 303-3s, New England Biolabs, Beverly, MA) is suitable for most Gram-negative bacteria while various origins from SV40, polyoma, adenovirus, vesicular stomatitis virus (VSV) or papillomaviruses (such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it contains the early promoter).

A transcription termination sequence is typically located 3' of the end of a polypeptide coding regions and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment followed by a poly T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector,

it can also be readily synthesized using methods for nucleic acid synthesis such as those described above.

A selectable marker gene element encodes a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells, (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex media. Preferred selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. A neomycin resistance gene may also be used for selection in prokaryotic and eukaryotic host cells.

Other selection genes may be used to amplify the gene which will be expressed. Amplification is the process wherein genes which are in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of the marker present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection partner in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes an antibody. As a result, increased quantities of an antibody are synthesized from the amplified DNA.

A ribosome binding site is usually necessary for translation initiation of mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically

located 3' to the promoter and 5' to the coding sequence of the polypeptide to be expressed. The Shine-Dalgarno sequence is varied but is typically a polypurine (i.e., having a high A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be readily synthesized
5 using methods set forth above and used in a prokaryotic vector.

A leader, or signal, sequence is used to direct secretion of a polypeptide. A signal sequence may be positioned within or directly at the 5' end of a polypeptide coding region. Many signal sequences have been identified and may be selected based upon the host cell used for
10 expression. In the present invention, a signal sequence may be homologous (naturally occurring) or heterologous to a nucleic acid sequence encoding an antibody or antigen binding domain. A heterologous signal sequence selected should be one that is recognized and processed, i.e., cleaved, by a signal peptidase, by the host cell. For
15 prokaryotic host cells that do not recognize and process a native immunoglobulin signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or heat-stable enterotoxin II leaders. For yeast secretion, a native immunoglobulin signal sequence may be
20 substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

In most cases, secretion of an antibody or antigen binding domain from a host cell will result in the removal of the signal peptide from the
25 antibody. Thus the mature antibody will lack any leader or signal sequence.

In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various presequences to improve glycosylation or yield. For example, one may

alter the peptidase cleavage site of a particular signal peptide, or add prosequences, which also may affect glycosylation. The final protein product may have, in the -1 position (relative to the first amino acid of the mature protein) one or more additional amino acids incident to
5 expression, which may not have been totally removed. For example, the final protein product may have one or two amino acid found in the peptidase cleavage site, attached to the N-terminus. Alternatively, use of some enzyme cleavage sites may result in a slightly truncated form of the desired polypeptide, if the enzyme cuts at such area within the mature
10 polypeptide.

The expression vectors of the present invention will typically contain a promoter that is recognized by the host organism and operably linked to a nucleic acid molecule encoding an antibody or antigen binding domain. Either a native or heterologous promoter may be used depending
15 the host cell used for expression and the yield of protein desired.

Promoters suitable for use with prokaryotic hosts include the beta-lactamase and lactose promoter systems; alkaline phosphatase, a tryptophan (trp) promoter system; and hybrid promoters such as the tac promoter. Other known bacterial promoters are also suitable. Their
20 sequences have been published, thereby enabling one skilled in the art to ligate them to the desired DNA sequence(s), using linkers or adapters as needed to supply any required restriction sites.

Suitable promoters for use with yeast hosts are also well known in the art. Yeast enhancers are advantageously used with yeast promoters.
25 Suitable promoters for use with mammalian host cells are well known and include those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40). Other

suitable mammalian promoters include heterologous mammalian promoters, e.g., heat-shock promoters and the actin promoter.

Additional promoters which may be used for expressing the specific binding partners of the invention include, but are not limited to:

5 the SV40 early promoter region (Benoist and Chambon (1981), Nature, 290:304-310); the CMV promoter; the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al. (1980), Cell, 22: 787-97); the herpes thymidine kinase promoter (Wagner et al. (1981), Proc. Natl. Acad. Sci. U.S.A., 78: 1444-5); the regulatory sequences of the

10 metallothioneine gene (Brinster et al. (1982), Nature, 296: 39-42); prokaryotic expression vectors such as the beta -lactamase promoter (Villa-Kamaroff et al. (1978), Proc. Natl. Acad. Sci. U.S.A., 75: 3727-31); or the tac promoter (DeBoer, et al. (1983), Proc. Natl. Acad. Sci. U.S.A., 80: 21-25). Also of interest are the following animal transcriptional control

15 regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region which is active in pancreatic acinar cells (Swift et al. (1984), Cell, 38: 639-46; Ornitz et al. (1986), Cold Spring Harbor Symp. Quant. Biol. 50: 399-409; MacDonald (1987), Hepatology, 7: :425-515); the insulin gene control region which is

20 active in pancreatic beta cells (Hanahan (1985), Nature, 315: 115-122); the immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al. (1984), Cell, 38: 647-58; Adames et al. (1985), Nature, 318: 533-8; Alexander et al. (1987), Mol. Cell. Biol., 7: 1436-44); the mouse mammary tumor virus control region which is active in testicular, breast,

25 lymphoid and mast cells (Leder et al. (1986), Cell, 45: 485-95), albumin gene control region which is active in liver (Pinkert et al. (1987), Genes and Devel., 1: 268-76); the alphafetoprotein gene control region which is active in liver (Krumlauf et al. (1987), Mol. Cell. Biol., 5: 1639-48; Hammer et al. (1987), Science, 235: 53-58); the alpha 1-antitrypsin gene control region

which is active in the liver (Kelsey *et al.* (1987), *Genes and Devel.*, 1: 161-171); the beta-globin gene control region which is active in myeloid cells (Mogam *et al.* (1985), *Nature*, 315: 338-340; Kollias *et al.* (1986), *Cell*, 46: '89-94); the myelin basic protein gene control region which is active in
5 oligodendrocyte cells in the brain (Readhead *et al.* (1987), *Cell*, 48: 703-712); the myosin light chain-2 gene control region which is active in skeletal muscle (Sani (1985), *Nature*, 314: 283-286); and the gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.* (1986), *Science*, 234: 1372-8).

10 An enhancer sequence may be inserted into the vector to increase transcription in eucaryotic host cells. Several enhancer sequences available from mammalian genes are known (e.g., globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus will be used. The SV40 enhancer, the cytomegalovirus early
15 promoter enhancer, the polyoma enhancer, and adenovirus enhancers are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be spliced into the vector at a position 5' or 3' to the polypeptide coding region, it is typically located at a site 5' from the promoter.

20 Preferred vectors for practicing this invention are those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen Company, San Diego, CA), pBSII (Stratagene Company, La Jolla, CA), pET15 (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2
25 (Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), pDSR-alpha (PCT Publication No. WO90/14363) and pFastBacDual (Gibco/BRL, Grand Island, NY).

Additional possible vectors include, but are not limited to, cosmids, plasmids or modified viruses, but the vector system must be compatible

with the selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript[®] plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene Cloning Systems Inc., La Jolla CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products
5 (e.g., TOPO[™] TA Cloning[®] Kit, PCR2.1[®] plasmid derivatives, Invitrogen, Carlsbad, CA), and mammalian, yeast or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo Alto, CA). The recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, or other
10 known techniques.

Host cells of the invention may be prokaryotic host cells (such as E. coli) or eukaryotic host cells (such as a yeast cell, an insect cell, or a vertebrate cell). Prokaryotic host cells such as E. coli produce unglycosylated protein; for example, unglycosylated shBCMA and
15 unglycosylated shTACI, which may possess advantages over the glycosylated eukaryotic molecules. The host cell, when cultured under appropriate conditions, expresses an antibody or antigen binding domain of the invention which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the
20 host cell producing it (if it is not secreted). Selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity, such as glycosylation or phosphorylation, and ease of folding into a biologically active molecule.

25 A number of suitable host cells are known in the art and many are available from the American Type Culture Collection (ATCC), Manassas, VA. Examples include mammalian cells, such as Chinese hamster ovary cells (CHO) (ATCC No. CCL61) CHO DHFR- cells (Urlaub et al. (1980), Proc. Natl. Acad. Sci. USA 97, 4216-20), human embryonic kidney (HEK)

293 or 293T cells (ATCC No. CRL1573), or 3T3 cells (ATCC No. CCL92). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Other suitable mammalian cell lines, are the monkey COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No. CRL1651), and the CV-1 cell line (ATCC No. CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines, which are available from the American Type Culture Collection, Manassas, VA). Each of these cell lines is known by and available to those skilled in the art of protein expression.

Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of E. coli (e.g., HB101, (ATCC No. 33694) DH5 α , DH10, and MC1061 (ATCC No. 53338)) are well-known as host cells in the field of biotechnology. Various strains of Pseudomonas spp., B. subtilis, other Bacillus spp., Streptomyces spp., and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention. Preferred yeast cells include, for example, Saccharomyces cerevisiae.

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described for

example in Kitts et al. (1993), Biotechniques, 14: 810-7, Lucklow (1993), Curr. Opin. Biotechnol., 4: 564-72, and Lucklow et al. (1993), J. Virol., 67: 4566-79. Preferred insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).

Transformation or transfection of a nucleic acid molecule encoding
5 a specific binding partner into a selected host cell may be accomplished by well known methods including methods such as calcium chloride, electroporation, microinjection, lipofection or the DEAE-dextran method. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the
10 skilled artisan, and are set forth, for example, in Sambrook et al., supra.

One may also use transgenic animals to express glycosylated specific binding partners, such as antibodies and antigen binding domain. For example, one may use a transgenic milk-producing animal (a cow or goat, for example) and obtain glycosylated binding partners in the animal
15 milk. Alternatively, one may use plants to produce glycosylated specific binding partners.

Host cells comprising (as by transformation or transfection) an expression vector encoding a specific binding partner of the target molecule may be cultured using standard media well known to the skilled
20 artisan. The media will usually contain all nutrients necessary for the growth and survival of the cells. Suitable media for culturing E. coli cells are for example, Luria Broth (LB) and/or Terrific Broth (TB). Suitable media for culturing eukaryotic cells are RPMI 1640, MEM, DMEM, all of which may be supplemented with serum and/or growth factors as
25 required by the particular cell line being cultured. A suitable medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin hydrolysate, and/or fetal calf serum as necessary.

Typically, an antibiotic or other compound useful for selective growth of transfected or transformed cells is added as a supplement to the

media. The compound to be used will be dictated by the selectable marker element present on the plasmid with which the host cell was transformed. For example, where the selectable marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin. Other compounds for selective growth include ampicillin, tetracycline and neomycin.

The amount of an antibody or antigen binding domain produced by a host cell can be evaluated using standard methods known in the art. Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, HPLC separation, immunoprecipitation, and/or activity assays.

Purification of a specific binding partner that has been secreted into the cell media can be accomplished using a variety of techniques including affinity, immunoaffinity or ion exchange chromatography, molecular sieve chromatography, preparative gel electrophoresis or isoelectric focusing, chromatofocusing, and high pressure liquid chromatography. For example, antibodies comprising a Fc region may be conveniently purified by affinity chromatography with Protein A, which selectively binds the Fc region. Modified forms of an antibody or antigen binding domain may be prepared with affinity tags, such as hexahistidine or other small peptide such as FLAG (Eastman Kodak Co., New Haven, CT) or myc (Invitrogen) at either its carboxyl or amino terminus and purified by a one-step affinity column. For example, polyhistidine binds with great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of polyhistidine-tagged specific binding partners. See for example, Ausubel et al., eds. (1993), Current Protocols in Molecular Biology, Section 10.11.8, John Wiley & Sons, New York. In some instances, more than one purification step may be required.

Specific binding partners of the invention which are expressed in procaryotic host cells may be present in soluble form either in the periplasmic space or in the cytoplasm or in an insoluble form as part of intracellular inclusion bodies. Specific binding partners can be extracted
5 from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by centrifugation.

Soluble forms of an antibody or antigen binding domain present
10 either in the cytoplasm or released from the periplasmic space may be further purified using methods known in the art, for example Fab fragments are released from the bacterial periplasmic space by osmotic shock techniques.

If an antibody or antigen binding domain has formed inclusion
15 bodies, they can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material can then be treated at pH extremes or with chaotropic partner such as a detergent, guanidine, guanidine derivatives, urea, or urea derivatives in the presence of a reducing partner such as
20 dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. The soluble specific binding partner can then be analyzed using gel electrophoresis, immunoprecipitation or the like. If it is desired to isolate a solubilized antibody or antigen binding domain, isolation may be accomplished using
25 standard methods such as those set forth below and in Marston et al. (1990), Meth. Enz., 182: 264-75.

In some cases, an antibody or antigen binding domain may not be biologically active upon isolation. Various methods for "refolding" or converting the polypeptide to its tertiary structure and generating

disulfide linkages, can be used to restore biological activity. Such methods include exposing the solubilized polypeptide to a pH usually above 7 and in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the choices used for inclusion
5 body solubilization, but usually the chaotrope is used at a lower concentration and is not necessarily the same as chaotropes used for the solubilization. In most cases the refolding/oxidation solution will also contain a reducing partner or the reducing partner plus its oxidized form in a specific ratio to generate a particular redox potential allowing for
10 disulfide shuffling to occur in the formation of the protein's cysteine bridge(s). Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol(DTT)/dithiane DTT, and 2-mercaptoethanol(bME)/dithio-
b(ME). In many instances, a cosolvent may be used or may be needed to
15 increase the efficiency of the refolding and the more common repartners used for this purpose include glycerol, polyethylene glycol of various molecular weights, arginine and the like.

Specific binding partners of the invention may also be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using
20 techniques known in the art such as those set forth by Merrifield et al. (1963), J. Am. Chem. Soc., 85: 2149; Houghten et al. (1985), Proc Natl Acad. Sci. USA, 82: 5132; and Stewart and Young (1984), Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL. Such polypeptides may be synthesized with or without a methionine on the amino terminus.
25 Chemically synthesized antibodies and antigen binding domains may be oxidized using methods set forth in these references to form disulfide bridges. Antibodies so prepared will retain at least one biological activity associated with a native or recombinantly produced antibody or antigen binding domain.

The invention will now be further described by specific experimental examples. These examples are meant to be illustrative rather than limiting.

Working Examples

5 Materials and Methods

Isolation of BCMA and TACI cDNA

Mouse and human BCMA cDNA were isolated by PCR using the mouse BCMA sense primer

5'-CACAATACCTGTGGCCCTCTTAAGAG-3'

10 (SEQ ID NO:25),

and antisense primer

5'-TGGTAAACGGTCATCCTAACGACATC-3'

(SEQ ID NO:26),

the human BCMA sense primer

15 5'-TTACTTGTCCTTCCAGGCTGTTCT-3'

(SEQ ID NO: 27),

and antisense primer

5'-CATAGAAACCAAGGAAGTTTCTACC-3'

(SEQ ID NO: 28).

20 For isolation of human TACI cDNA, the sense primer

5'-AGCATCCTGAGTAATGAGTGGCCTGG-3'

(SEQ ID NO: 29)

and antisense primer

5'-GTGATGACGACCTACAGCTGCACTGGG-3'

25 (SEQ ID NO: 30)

were used. Poly (A)+ RNA from the mouse B lymphoma cell line -A20 and human lymph Node were reverse -transcribed and cDNA were synthesized by using the Smart RACE cDNA amplification Kit (Clontech , palo Alto, California). The full-length cDNA of mouse and human BCMA

genes as well as human TACI gene were cloned into pcDNA3 vector for mammalian cell expression (Invitrogen, Carlsbad, California).

Recombinant proteins

Soluble murine APRIL-Flag protein was generated by fusing Flag
5 sequence in frame to the N-terminus of APRIL amino acid 101-239.

Soluble mAPRIL-Flag protein was expressed in *E. coli* and the refolded protein was affinity-purified by anti-Flag M2 antibody column. Fc-tagged AGP3 protein was generated by fusing OPG signal peptide followed by human IgG- γ 1 Fc in frame to the N-terminus of AGP3 amino acid 128-285.

10 The protein was expressed in baculovirus and purified with protein A sepharose column. Fc-tagged human APRIL was encoded by a similar construct (see Figure 21) and expressed in CHO cells and purified with protein A sepharose column.

Soluble TACI protein (amino acids 1-165) and BCMA protein
15 (amino acid 4-55) followed by human IgG- γ 1 Fc in frame was expressed in *E. coli*. The inclusion bodies formed were solubilized. The refolded protein was purified by cation exchange chromatography.

Purification of human BCMA-Fc

Purification of human BCMA-Fc produced in recombinant *E. coli*
20 was initiated by solubilizing 53.3 g of a washed inclusion body preparation in a solution having a final composition of 6M guanidinium chloride, 50mM Tris(HCl), 8.0 mM dithiothreitol. The final volume of solution was approximately 200mL. The solution was adjusted to pH 9.0 at 23 degrees Centigrade and allowed to stir at room temperature for one
25 hour.

The solubilized polypeptide solution was then added to 3.8 L of cold 4 M urea, 160 mM L-arginine, 20% (volume) glycerol, 4 mM cysteine, 1 mM cystamine, 50 mM Tris. The pH was adjusted to 8.9 at 12 degrees

Centigrade and the mixture was allowed to stir at 4-8 for approximately 60 hours.

The refolding mixture was then clarified by filtration through a 0.9 square foot Cuno 10SP cartridge. The filtrate was then concentrated
5 approximately five fold and diafiltered with five retentate volumes of 10 mM sodium phosphate/40 mM sodium chloride, pH 9 using a 1.0 square foot Pall Filtron regenerated cellulose TFF cassette having a nominal molecular weight cutoff of 50 kDa. Processing was performed at 4-8 degrees Centigrade.

10 The retentate was removed from the unit, warmed to 20 degrees Centigrade, and adjusted to pH 5.0 using 1 M acetic acid. The precipitated solids were then removed by centrifugation in a Beckman J6-B centrifuge operating at 20 degrees Centigrade at approximately 2500 x g for approximately 15 minutes. The supernatant was aliquotted and stored
15 frozen at -30 degrees Centigrade.

Aliquots of supernatant were thawed and processed over a 5.0 cm diameter x 27.5 cm height column of SP-Sepharose Fast Flow. All phases of the chromatography were performed at 8 degrees Centigrade. The column was equilibrated using 25mM sodium phosphate, pH 6.5. The
20 product was conditioned for loading by adjusting the pH to 6.5. Following the load, the column was washed with equilibration buffer, and the product was then eluted with a linear gradient from 17 mM to 60 mM sodium chloride in a background of 25 mM sodium phosphate pH 6.5, over 20 column volumes. Fractions were selected for further processing
25 based on their appearance on a Coomassie blue-stained SDS polyacrylamide gel.

The pooled SP-Sepharose Fast Flow fractions were further processed over a 5.0 cm diameter x 27.5 cm height column of Butyl Toyopearl 650M, operated at 21 degrees Centigrade. The product was

conditioned prior to loading by adding solid potassium phosphate to give a final concentration of approximately 0.6 M. The column was equilibrated using 0.6M potassium phosphate, pH 7.0. After loading, the column was washed with 0.5 bed volumes of equilibration buffer, and the
5 product was then eluted using a linear gradient from 0.5 M to 0.3 M potassium phosphate over 15 column volumes. Fractions from the predominant UV elution peak were pooled for further processing.

The pooled fractions from the Butyl Toyopearl chromatography were concentrated twenty fold and diafiltered against six retentate
10 volumes of phosphate buffered saline using a Millipore XL TFF cassette, operated at 4-8 degrees Centigrade. The final retentate pool was aliquotted and stored at -30 degrees Centigrade.

In vivo study

B6 mice (6-8 weeks old) were purchased from Charles River
15 Laboratories and murine APRIL-Flag and other TNF proteins were injected i.p. of 1 mg/kg/day for 5 days. On day 7, cells from mouse spleens and mesenteric lymph nodes were collected and B and T cell activation and differentiation was analyzed by FACS using specific monoclonal antibodies staining.

20 **Cell lines and proliferation assays**

293 human kidney epithelial cells, Raji Burkitt lymphoma, human T lymphoblastoma Jurkat cells and A20, mouse B lymphoma cell line were purchased from the American Type Culture Collection (Rockville, Maryland). Raji, Jurkat and A20 cells were maintained in a complete
25 medium of RPMI-1640 (Life Technologies) supplemented with 10% fetal bovine serum (HyClone, Logan, Utah) and 25 mM HEPES. 293 cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies) with 10% fetal bovine serum. The proliferation of cells were determined by

incubating 5×10^4 cells /well in 100 μ L medium with the indicated concentration of APRIL-flag protein using the celltiter 96 AQ proliferation assay (Promega Corp.,Madison, WI) following the manufacturer's instructions. Alternatively, cells were pulsed for 18 h with ^3H thymidine
5 (0.5 μCi /well), after harvesting cells, ^3H thymidine incorporation was monitored by liquid scintillation counting.

Transfection and Flow cytometric analysis

For 293 cell expressing BCMA and TACI receptor, 2×10^6 293 cells were plated into 6 well plate, cells were transfected with lipofectAMINE
10 2000 following the manufacturer's procedure (Life Technologies), 48 h after transfection, cells were collected and incubated at 4 C with 1 $\mu\text{g}/\text{ml}$ APRIL-Flage ligand or Blys (AGP3)-Fc ligand for 60 min, after washing 3 times with PBS (containing 2% FBS), cells were stained with FITC-
15 and fluorescence was analyzed by FACS scanner (Becton Dickinson, Mountain View, California).

Determination of the binding affinities of APRIL and TALL-1 for BCMA and TACI

Biomolecular interaction analysis (BIA) was performed using a
20 BIACORE 2000 (Biacore AB, Uppsala, Sweden). The receptors, BCMA-Fc and TACI-Fc (2 $\mu\text{g}/\text{ml}$ in 10 mM sodium acetate, pH 4.5), were immobilized on Sensor Chip CM5 using the BIACORE standard amine coupling procedure. An immobilization level of approximately 120 RU's was achieved. The analytes, Flag-APRIL and Fc-AGP-3 were diluted
25 between 100 nM-0.01 nM in running buffer (10 mM HEPES, 0.5 M NaCl, 3 mM EDTA, 0.005% Tween 20, 2mg/ml CM dextran, pH 6.8). The analytes were injected over an immobilized receptor surface for 2 minutes at 50 $\mu\text{l}/\text{min}$ and allowed to dissociate for 10 minutes. Bound protein was removed by a 1minute injection of 50 mM HCl. Binding affinities were

determined using a 1:1 Langmuir model (BIA Evaluation software Version 3.1.2, BIACORE).

T cell co-stimulation assay

T cells from the spleens of C57 BL/6 mice were purified by negative
5 selection through a murine T cell enrichment column (R&D Systems). T
cells (1×10^5 per well) were cultured in the absence or presence of various
APRIL-Flag protein for 48 hr. Alternatively, 96 well plates were precoated
with subliminal quantities of anti-CD3 antibody, T cells were treated with
APRIL-Flag protein for 72 hr, pulsed during the last 18 hr with 1 μ Ci of 3 H
10 thymidine and harvested to count the incorporation radioactivity.

B cell proliferation and Ig secretion

Mouse B cell were negatively selected from spleens by mouse B cell
recovery column (Cedarlane, Hornby, Ontario Canada). 1×10^6 /ml were
seeded in 96 -well flat bottom tissue culture plates in medium (RPMI-
15 1640, 5% FBS, 5×10^5 M 2 ME, affinity-purified goat anti-mouse IgM 2.5
 μ g/ml Pharmingen, San Diego). B cells were then treated with APRIL -
Flag protein plus different concentration of soluble BCMA-Fc protein for
72 hr and culture received 1 μ Ci of 3 H thymidine during the last 18 hr.
proliferation of B cell was quantitated by measuring the incorporation of
20 radioactivity.

For analysis of Ig secretion from B cells, purified B cells 5×10^5 /ml
were cultured in 96-well flat bottom tissue culture plates in the presence
of APRIL-Flag for six days. The culture supernatant were harvested and
IgG, IgM and IgA levels were determined by an isotype specific sandwich
25 ELISA technique. Ig concentration in test samples were determined by
comparing triplicate test values with isotype control standard.

Induction and detection of anti-keyhole limpet hemocyanin (KLH) and anti-Pneumovax antibodies.

Mice (Balb/c females of 9-11 wk and 19-21 g, Charles River Laboratories, Wilmington, MA) were immunized on day 0 with 100 µg of KLH (Pierce, Rockford, IL) in CFA s.c. or with 115 µg of Pneumovax (Merck, West Point, PA) i.p. Starting on day 0, mice received 7 daily i.p. injections of 5 mg/Kg of either TACI-Fc or BCMA-Fc fusion proteins or non-fused Fc and were then bled on day 7. Anti-KLH and anti-Pneumovax IgG and IgM were measured in serum by ELISA. Briefly, for the measurement of anti-KLH antibodies, plates were coated with KLH in PBS, blocked, and added with dilutions of standard and test samples. Captured anti-KLH IgG or IgM were revealed using anti-IgG or anti-IgM biotinylated antibodies and neutravidin-conjugated HRP. For the measurement of anti-Pneumovax IgM, plates were coated with Pneumovax using poly-L-lysine, blocked, and added with dilutions of standard and test samples. Captured anti-Pneumovax IgM were revealed using an anti-IgM biotinylated antibody and neutravidin-conjugated HRP. Results were compared with the Student t test.

In a second experiment, normal mice (n=7) were treated with human BCMA-Fc, truncated TACI-Fc and nonfused Fc as a control in daily doses of 0.5mg/kg to 15 mg/kg for 7 days. At preimmunization anti-KLH and anti-Pneumovax were undetectable. Antibodies were measured on day 7 and Day 14. **hBCMA-Fc effect on mouse peripheral blood B cell in vivo.** Normal mice (15 mg/kg ip on day 0, 3, and 6; n=7) were treated with human BCMA-Fc and nonfused Fc as a control in day 0, 3, and 6 (15mg/kg). Peripheral blood and spleen B cell level were measured on day 7. **Flag-mAPRIL, hAGP3, hBCMA-Fc and hTACI-Fc effects on immunoglobulin production *in vitro*.** Purified murine spleen B cells were cultured with LPS (100ng/ml), AGP3 (10ng/ml), Flag-APRIL (10ng/ml) or plus BCMA-Fc (100ng/ml) and TACI-Fc (100ng/ml) for 12 days. Culture supernatants were collected on day 7 and day 12 for detecting IgA and

IgG levels. mBCMA-Fc and trun hTACI-Fc effect on immunoglobulin levels in vitro.

Normal mice were treated with mBCMA-Fc; trun hTACI-Fc; or Fc-control (5 mg/kg ip day 0, 3, and 6; n=7). Immunoglobulin levels in serum
5 were measured on day 7.

Generation and analysis of anti-mAPRIL specific monoclonal antibody.

Flag-mAPRIL was used as antigen for generation of rat anti-mAPRIL specific monoclonal antibodies following standard procedures. Fourty
10 clones that recognized Flag-APRIL in an ELISA were identified. The clones effect on Flag-mAPRIL mediated mouse B cell proliferation was determined. Purified murine spleen B cells were cultured in presence 10ng/ml mFlag-APRIL plus 2 ug/ml of anti-IgM . anti-Flag-APRIL monoclonal antibody and rat IgG control were added into culture in same
15 time. Data show incorporation of ³H thymidine as cpm, and represent mean of triplicate wells. Anti-mAPRIL specific monoclonal antibody #c19 5 mg/kg ip on day 0, 3, and 6 was used for determining the effect of blocking endogenous APRIL on anti-Pneumovacs IgM *In Vivo*.

NZBxNZWF1 lupus model.

20 Five months old NZBxNZWF1 mice were treated 3 times/week (mon, wed, fri) for a period of 5 months with the indicated amount of protein. 100 ug dose translates to 4mg/Kg. Mice were bled at day of treatment to later be analyzed for DNA-specific antibodies, histone proteins, etc. Also at day 0, urine were taken and analyzed for proteins
25 levels. Animals are excluded that already have high levels of protein in the urine, a sign of lupus. Treatment was injected i.p. Urine and blood are taken every 30 days and analyzed until day 150.

APRIL binding and stimulation of tumor cells.

APRIL binding to human tumor cell lines were determined by incubating cell with 1ug/ml Fc-APRIL or Flag-APRIL following FITC labeled secondary antibody staining and FACS analysis. APRIL and BCMA-Fc or TACI-Fc effect on U266-B1 cell growth: U266 cells were
5 cultured in presence 10ng/ml human Fc-APRIL or plus 50ng/ml soluble BCMA-Fc, Truncated TACI-Fc for 48 hr. Incorporation of ³H thymidine is indicated as cpm. Data shown represent mean of triplicate wells. A20 mouse B cell lymphoma were cultured in presence 50ng/ml mouse Flag-APRIL, human Flag-AGP3 or plus 100 ng/ml soluble BCMA-Fc for 48 hr.
10 Incorporation of ³H thymidine is indicated as cpm. Data shown represent mean of triplicate wells.

A20 B lymphoma tumor cell growth in Balb/c mice.

A20 (0.2 million) cells were implanted, id, on day 0. Treatments with PBS, CHO-Fc, mBCMA-Fc, hBCMA-Fc, mTACI-Fc, hTACI-Fc were
15 given (10 mg/kg, ip) on days 0, 7, 10, 13, 16, 19, 22. Tumor measurements were made twice per week. Mice were sacrificed on days 27-31, tumors were snap frozen for RNA isolation, blood was collected and serum samples were frozen.

Human colon carcinoma cell line HT29 tumor growth.

20 2×10^6 HT29 cells plus 50% matrigel injected subcutaneously into athymic nude mice. Rx: human or mouse BCMA-Fc at 2, 5, and 15 mg/kg Q2D, starting at day 0 (n=10/group). Control 1: CHO-Fc at 15 mg/kg Q2D. Control 2: 0.2 ml of PBS Q2D IP. Tumor volume: 3/week, from day 7. Tumor weight at end of study. Body weight 2/week.

25 **Results**

G70/APRIL in vitro function

Human and mouse G70, also called APRIL, was isolated and characterized (Figures 1, 2, and 3). FLAG-tagged soluble mouse G70 (smG70) was produced in E. coli purified and refolded (Figure 2). Soluble

G70 (smG70) specifically stimulates B and T cell lymphoma cell proliferation in a dose-dependent manner (Figure 4). Furthermore, soluble G70:

- 1) specifically binds to cell-surface receptors expressed on human B and T lymphoma cells (Figure 5);
- 2) specifically stimulates proliferation of purified human peripheral blood B and T cells (Figure 6);
- 3) stimulates proliferation of purified murine spleen B and T cells in a dose-dependent manner (Figure 7);
- 4) acts synergistically with anti-CD28 antibody to stimulate proliferation of purified murine T cells (Figure 8);
- 5) has a strong costimulatory activity on purified murine T cells (Figure 9) in the presence of sub-optimal concentration of the T cell receptor activator: anti-CD3 antibody.

G70/APRIL in vivo function

A series of experiments were performed to elucidate soluble G70/APRIL's biological activity in normal mice in vivo. Each group consisted of 5 mice (BDF-1, 8 weeks of age, dosed at 1 mg/kg/day, 0.2 ml for 5 days). Spleen, thymus and mesenteric lymph nodes from three mice of each group was used for FACS analysis using a panel of T cell and B cell surface marker antibodies and all the mice were analyzed by standard necropsy and pathological analysis.

Spleen (Table 1A): murine soluble G70 caused an average about 60% decrease in the percentage of CD3⁺ T cells. In addition, there was an average 5-fold increase in T-helper cells activation and an average 22-fold increase in cytotoxic T cell activation as measured by IL-2 receptor expression. In addition the percentage of immature B cells increased about 2-fold while the percentage of mature B-cells increased 3- to 4-fold.

The total percentage of lymphocytes (T+B) was unchanged compared to control.

Mesenteric Lymph Nodes (Table 1B): soluble G70 treated mice had an average of 25% decrease in the percentage of T cells. There was an
5 average 3-fold increase in % activated T-helper cells and 36-fold increase in activated cytotoxic T-cells as measured by CD25/IL-2 receptor expression. In addition the percentage of immature B cells was increased on average 2-fold whereas mature B cells were up on average 4-fold.

In summary our preliminary observations indicate that G70/APRIL
10 stimulates both T and B cells in the spleen and mesenteric lymph nodes. Pathological analysis revealed that soluble G70 treated mice have slightly enlarged spleens of normal morphology.

G70/APRIL is a ligand for BCMA and TACI

G70/APRIL is related to the TNF ligand family member
15 AGP3/BlyS. The TNFR receptor family member TACI (Fig.12) was recently shown to be a receptor for AGP3 ([A-570A patent application ser. no.]). Furthermore, TACI has a match to the orphan TNFR receptor family member BCMA (Figure 10) in a conserved extracellular cysteine rich domain (Figure 13). These observations together prompted us to
20 investigate whether G70/APRIL is a ligand for BCMA and TACI and to test whether in addition to TACI AGP3/BlyS is also a ligand for BCMA.

Soluble mouse G70 specifically binds to 293 cells expressing exogenous BCMA (Figure 14). G70 also binds to 293 cells expressing TACI (Figure 15). Furthermore soluble G70 specifically blocks AGP3/BlyS
25 binding to cell-surface receptors located on mouse B lymphoma cells (Figure 16). This suggest that G70 and AGP3 both binds to BCMA and TACI.

smBCMA-Fc and shTACI-Fc prevent G70 and AGP3 ligand binding to cell-surface receptors

Soluble BCMA (smBCMA-Fc; Fig. 10) and soluble TACI (shTACI-Fc) were produced in *E. coli* purified to homogeneity and refolded.

Soluble TACI receptor specifically prevents G70 from binding to mouse B cells. (Figure 17). Furthermore, shBCMA-Fc and shTACI-Fc both
5 prevent binding of AGP3 to B cells (Figure 18; and Figure 19 A). Soluble hBCMA-Fc also ameliorates G70 binding to A20 cells (Figure 19 B).

In summary: 1) both G70 and AGP3 binds the orphan TNFR receptor family members TACI and BCMA; 2) soluble BCMA and TACI both effectively inhibits G70 and AGP3 from binding to B cells; 3) G70 and
10 AGP3 competes for binding to cell-surface receptors.

Effects of TACI-Fc and BCMA-Fc treatment on the production of anti-KLH and anti-Pneumovax antibodies.

Treatment with either TACI-Fc or BCMA-Fc significantly inhibited the production of anti-KLH and anti-Pneumovax antibodies. Serum levels
15 of both anti-KLH IgG and IgM were approximately 25% and 19% lower, respectively, in the TACI-Fc-treated mice than controls (Figure 20). Serum anti-KLH IgG and IgM were approximately 52% and 66% lower, respectively, in the BCMA-Fc-treated mice than controls (Figure 20). Serum levels of anti-Pneumovax IgM were also lower in the TACI-Fc- and
20 BCMA-Fc-treated mice than controls (24% and 42%, respectively, Figure 20). Second experiment: Treatment with hBCMA-Fc or Trun-hTACI-Fc reduces anti-Pneumovacs specific IgM levels in normal mice (Figure 29). Normal mice (n=7) were treated with human BCMA-Fc, truncated TACI-Fc and nonfused Fc as a control in daily doses of 0.5mg/kg to 15 mg/kg for 7
25 days. At preimmunization anti-KLH and anti-Pneumovax were undetectable. Antibodies were measured on day 7 and Day 14.

Effects of hBCMA-Fc on progression of lupus in NZBXNZWF1 mice.

Treatment of NZB/NXWF1 mice with human BCMA-Fc increased survival, decreased proteinuria, decreased the level of anti-dsDNA specific antibodies, and decreased % B cells in peripheral blood. Protocol for SLE: Five months old NZBxNZWF1 mice were treated 3 times/week (mon, wed, fri) for a period of 5 months with the indicated amount of protein. 100 ug dose translates to 4mg/Kg. Mice were bled at day of treatment to later be analyzed for DNA-specific antibodies, histone proteins, etc. Also at day 0, urine were taken and analyzed for proteins levels. Animals are excluded that already have high levels of protein in the urine, a sign of lupus. Treatment was injected i.p. Urine and blood are taken every 30 days and analyzed until day 150.

Effects of hBCMA-Fc on A20 lymphoma cell growth in vivo.

Treatment with BCMA-Fc reduces A20 B lymphoma tumor cell growth in Balb/c mice. A20 (0.2 million) cells were implanted, id, on day 0. Treatments with PBS, CHO-Fc, mBCMA-Fc, hBCMA-Fc, mTACI-Fc, hTACI-Fc were given (10 mg/kg, ip) on days 0, 7, 10, 13, 16, 19, 22. Tumor measurements were made twice per week. Mice were sacrificed on days 27-31, tumors were snap frozen for RNA isolation, blood was collected and serum samples were frozen.

Effects of hBCMA-Fc on human colon carcinoma HT29 cell growth in vivo.

Treatment with hBCMA-Fc reduces human colon carcinoma cell line HT29 tumor volume growth in mice. 2×10^6 cells plus 50% matrigel injected subcutaneously into athymic nude mice. Rx: human BCMA-Fc at 2, 5, and 15 mg/kg Q2D, starting at day 0 (n=10/group). Control 1: CHO-Fc at 15 mg/kg Q2D. Control 2: 0.2 ml of PBS Q2D IP. Tumor volume: 3/week, from day 7. Tumor weight at end of study. Body weight 2/week.

Fc-APRIL effect on B cells

Fc-humanAPRIL and soluble humanAGP3/BlyS/Tall-1 stimulates incorporation of ³H thymidine in primary murine B cells. Purified murine spleen B cells were cultured in presence of various amounts of human Fc-
5 APRIL and untaged AGP3 plus 2 ug/ml of anti-IgM . Data show incorporation of ³H thymidine as cpm, and represent mean of triplicate wells.

hBCMA-Fc effect on APRIL mediated B cell proliferation

hBCMA-Fc and hTACI-Fc inhibits Flag-mAPRIL mediated mouse B cell
10 proliferation (Figure 23). Purified murine spleen B cells were cultured with the indicated amounts of soluble BCMA-Fc and TACI-Fc in presence of 10 ng/ml of Flag-mAPRIL and 2 ug/ml of an anti-IgM for 72 hr. Incorporation of ³H thymidine is indicated as cpm. Data shown represent mean of triplicate wells.

15 **hBCMA-Fc effect on peripheral blood B cell level** hBCMA-Fc (15 mg/kg ip on day 0, 3, and 6) reduces mouse peripheral blood and B cell levels measured at day seven (Figure 24 and 25). Normal mice (n=7) were treated with human BCMA-Fc and nonfused Fc as a control in day 0,3, and 6 (15mg/kg). Peripheral blood and spleen B cell (B220) level were
20 measured on day 7.

Flag-mAPRIL and hAGP3 mediated IgA production is inhibited by hBCMA-Fc and hTACI-Fc *in vitro* (Figure 26. Purified murine spleen B cells were cultured with LPS (100ng/ml), AGP3 (10ng/ml), Flag-APRIL (10ng/ml) or plus BCMA-Fc (100ng/ml) and TACI-Fc (100ng/ml) for 12
25 days. Culture supernatants were collected on day 7 and day 12 for detecting IgA level. Flag-mAPRIL and hAGP3 mediated IgG production is inhibited by hBCMA-Fc and hTACI-Fc *in vitro* (Figure 27).

BCMA-Fc effect on total immunoglobulin in vivo.

Total IgE and IgA levels are reduced in normal mice treated with mBCMA-Fc and trun hTACI-Fc (5 mg/kg *ip day 0, 3, and 6*). Normal mice (n=7) were treated with human BCMA-Fc ,Truncated TACI-Fc and nonfused Fc as a control in day 0,3, and 6 (5mg/kg). Immunoglobulin
5 levels in serum were measured on day 7.

anti-mAPRIL specific monoclonal antibody effect.

The anti-mAPRIL specific monoclonal antibody #c19 inhibits Flag-mAPRIL mediated mouse B cell proliferation (Figure 30). Purified murine spleen B cells were cultured in presence 10ng/ml mFlag-APRIL plus 2
10 ug/ml of anti-IgM . anti-Flag-APRIL monoclonal antibody c-19 and rat IgG control were added into culture in same time. Data show incorporation of 3H thymidine as cpm,and represent mean of triplicate well2. Treatment with the anti-mAPRIL specific monoclonal antibody #c19 inhibits generation of anti-Pneumovacs specific antibodies *in vivo*
15 (Figure 31).

APRIL binding and effect on tumor cells.

APRIL binds to a number of tumor cell lines (Fig 36). APRIL binding to human tumor cell lines were determined by incubating cell with 1ug/ml Fc-APRIL or Flag-APRIL following FITC labeled secondary
20 antibody staining and FACS analysis. APRIL stimulates U266-B1 cell growth and that this can be inhibited by BCMA-Fc or TACI-Fc (Figure 37). U266 cells were cultured in presence 10ng/ml human Fc-APRIL or plus 50ng/ml soluble BCMA-Fc, Truncated TACI-Fc for 48 hr. Incorporation of 3H thymidine is indicated as cpm. Data shown represent mean of
25 triplicate wells.

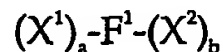
What is claimed is:

1. A method of inhibiting B or T cell proliferation or activation in a
5 mammal, which comprises administering a therapeutic agent
comprising:
 - a. a specific binding partner for TACI, wherein the specific binding
partner has TACI antagonist activity;
 - b. a specific binding partner for BCMA, wherein the specific binding
10 partner has BCMA antagonist activity;
 - c. both a and b; or
 - d. a specific binding partner for TACI and BCMA, wherein the
specific binding partner has TACI antagonist activity, BCMA
antagonist activity or both.
- 15 2. A method of inhibiting APRIL activity in a mammal, which comprises
administering a therapeutic agent comprising:
 - a. a specific binding partner for TACI, wherein the specific binding
partner has TACI antagonist activity;
 - b. a specific binding partner for BCMA, wherein the specific binding
20 partner has BCMA antagonist activity;
 - c. both a and b; or
 - d. a specific binding partner for TACI and BCMA, wherein the
specific binding partner has TACI antagonist activity, BCMA
antagonist activity or both.
- 25 3. A method of inhibiting TACI activity, BCMA activity, or both in a
mammal, which comprises administering a specific binding partner for
APRIL.
4. The method of Claim 3, further comprising administering a specific
binding partner for AGP-3.

5. A method of increasing T cell proliferation in a mammal, which comprises administering a therapeutic agent comprising:
- a. a specific binding partner for TACI, wherein the specific binding partner has TACI agonist activity;
 - 5 b. a specific binding partner for BCMA, wherein the specific binding partner has BCMA agonist activity;
 - c. both a and b; or
 - d. a specific binding partner for TACI and BCMA, wherein the specific binding partner has TACI agonist activity, BCMA agonist
10 activity or both.
6. A method of increasing APRIL activity in a mammal, which comprises administering a therapeutic agent comprising:
- a. a specific binding partner for TACI, wherein the specific binding partner has TACI agonist activity;
 - 15 b. a specific binding partner for BCMA, wherein the specific binding partner has BCMA agonist activity;
 - c. both a and b; or
 - d. a specific binding partner for TACI and BCMA, wherein the specific binding partner has TACI agonist activity, BCMA agonist
20 activity or both.
7. A method of treating B-cell lymphoproliferative disorders, which comprises administering a therapeutic agent comprising an amino acid sequence selected from:
- a. the extracellular region of TACI (SEQ ID NO: 15);
 - 25 b. the extracellular region of BCMA (SEQ ID NO: 6);
 - c. the consensus region of TACI (SEQ ID NO: 16);
 - d. the consensus region of BCMA (SEQ ID NO: 7);
 - e. the TACI/BCMA extracellular consensus sequence (SEQ ID NO: 13).

8. A method of treating T-cell lymphoproliferative disorders, which comprises administering a therapeutic agent comprising an amino acid sequence selected from selected from:
- a. the extracellular region of TACI (SEQ ID NO: 15);
 - 5 b. the extracellular region of BCMA (SEQ ID NO: 6);
 - c. the consensus region of TACI (SEQ ID NO: 16);
 - d. the consensus region of BCMA (SEQ ID NO: 7);
 - e. the TACI/BCMA extracellular consensus sequence (SEQ ID NO: 13)..
- 10 9. A method of treating one or more solid tumors, which comprises administering a therapeutic agent comprising an amino acid sequence selected from selected from:
- a. the extracellular region of TACI (SEQ ID NO: 15);
 - b. the extracellular region of BCMA (SEQ ID NO: 6);
 - 15 c. the consensus region of TACI (SEQ ID NO: 16);
 - d. the consensus region of BCMA (SEQ ID NO: 7);
 - e. the TACI/BCMA extracellular consensus sequence (SEQ ID NO: 13)..
- 20 10. The method of Claim 9, wherein the tumor is selected from lung, gastrointestinal, pancreatic and prostate
11. The method of any of Claims 1 to 6, wherein the specific binding partner is an antibody.
12. The method of Claim 11, wherein the antibody is a monoclonal antibody.
- 25 13. The method of Claim 11, wherein the antibody is a fully human antibody, a humanized antibody, or an antibody derived from a phage display library.
14. The methods of any of Claims 1 to 6, wherein the specific binding partner is a peptide.

15. The method of Claim 14, wherein the specific binding partner is comprised within a molecule of the formula



wherein:

5 F^1 is a vehicle;

X^1 and X^2 are each independently selected from $-(L^1)_c-P^1$, $-(L^1)_c-P^1-(L^2)_d-P^2$, $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$, and $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$

P^1 , P^2 , P^3 , and P^4 are each independently peptide sequences, wherein at least one is a specific binding partner;

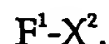
10 L^1 , L^2 , L^3 , and L^4 are each independently linkers; and

a , b , c , d , e , and f are each independently 0 or 1, provided that at least one of a and b is 1.

16. The method of Claim 15, wherein the molecule comprises a structure of the formulae



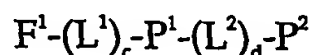
or



17. The method of Claim 15, wherein the molecule comprises a structure of the formula



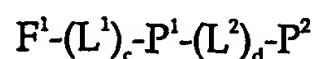
18. The method of Claim 15, wherein the molecule comprises a structure of the formula



wherein one of P^1 and P^2 is a specific binding partner for TACI and the other is a specific binding partner for BCMA.

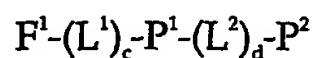
25

19. The method of Claim 15, wherein the molecule comprises a structure of the formula



wherein one of P¹ and P² is a specific binding partner for APRIL and the other is a specific binding partner for AGP-3.

20. The method of Claim 15 of the formula



5 wherein one of P¹ and P² is a specific binding partner for APRIL and the other is a specific binding partner for AGP-3.

21. The method of Claim 15, wherein the vehicle is an Fc domain.

22. The method of Claim 3, wherein the specific binding partner comprises a sequence selected from:

- 10 a. the extracellular region of TACI (SEQ ID NO: 15).
- b. the extracellular region of BCMA (SEQ ID NO: 6).
- c. the consensus region of TACI (SEQ ID NO: 16).
- d. the consensus region of BCMA (SEQ ID NO: 7).
- e. the TACI/BCMA extracellular consensus sequence (SEQ ID NO: 13).

23. The method of any of Claims 7, 8, 9 and 22, wherein the specific binding partner is covalently linked to a vehicle.

24. The method of Claim 23, wherein the vehicle is an Fc domain.

25. The method of Claim 3, wherein the specific binding partner is
20 comprised within a molecule having an antibody sequence in which one or more antibody CDR regions are replaced by one or more sequences selected from:

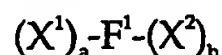
- a. the extracellular region of TACI (SEQ ID NO: 15);
- b. the extracellular region of BCMA (SEQ ID NO: 6);
- 25 c. the consensus region of TACI (SEQ ID NO: 16);
- d. the consensus region of BCMA (SEQ ID NO: 7);
- e. the TACI/BCMA extracellular consensus sequence (SEQ ID NO: 13);

f. the sequence of a peptide capable of specifically binding APRIL;
and

g. the sequence of a peptide capable of specifically binding AGP-3.

26. The method of any of Claims 7, 8, and 9, wherein said amino acid
5 sequence replaces a CDR region within an antibody molecule.

27. A composition of matter of the formula



wherein:

F^1 is a vehicle;

10 X^1 and X^2 are each independently selected from $-(L^1)_c-P^1$, $-(L^1)_c-P^1-(L^2)_d-P^2$, $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$, and $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$
 P^1 , P^2 , P^3 , and P^4 are each independently peptide sequences, wherein
at least one is a specific binding partner for TACI, BCMA, or APRIL;
 L^1 , L^2 , L^3 , and L^4 are each independently linkers; and

15 a, b, c, d, e, and f are each independently 0 or 1, provided that at
least one of a and b is 1.

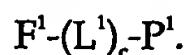
28. The composition of matter of Claim 27 of the formulae



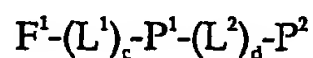
or

20 F^1-X^2 .

29. The composition of matter of Claim 27 of the formula



30. The composition of matter of Claim 27 of the formula



25 wherein one of P^1 and P^2 is a specific binding partner for TACI and the
other is a specific binding partner for BCMA.

31. The composition of matter of Claim 27, wherein the vehicle is an Fc
domain.

32. An isolated nucleic acid encoding the composition of matter of Claim 31.
33. The nucleic acid of Claim 32 including one or more codons preferred for Escherichia coli expression.
- 5 34. An expression vector comprising the nucleic acid of Claim 32.
35. A host cell transformed or transfected with the expression vector of Claim 34.
36. The host cell of Claim 35, wherein the cell is a prokaryotic cell.
37. The host cell of Claim 36, wherein the cell is Escherichia coli.

10

Figure 1

Sequence of human APRIL (SEQ ID NOS: 1 and 2)

Human G70 cDNA (SEQ ID NO 1)

Length: 1465 bp

```

1  GCCAACCTTC CCTCCCCCAA CCCTGGGGCC GCCCCAGGGT TCCTGCGCAC
51  TGCCTGTTCC TCCTGGGTGT CAGTGGCAGC CCTGTCTTC CTAGAGGGAC
101 TGGAACCTAA TTCTCCTGAG GCTGAGGGAG GGTGGAGGGT CTCAAGGCAA
151 CGCTGGCCCC ACGACGGAGT GCCAGGAGCA CTAACAGTAC CCTTAGCTTG
201 CTTTCCTCCT CCCTCCTTTT TATTTTCAAG TTCCTTTTTA TTTCTCCTTG
251 CGTAACAACC TTCTTCCCTT CTGCACCACT GCCCGTACCC TTACCCGCCC
301 CGCCACCTCC TTGCTACCCC ACTCTTGAAA CCACAGCTGT TGGCAGGGTC
351 CCCAGCTCAT GCCAGCCTCA TCTCCTTTCT TGCTAGCCCC CAAAGGGCCT
401 CCAGGCAACA TGGGGGGCCC AGTCAGAGAG CCGGCACTCT CAGTTGCCCT
451 CTGGTTGAGT TGGGGGGCAG CTCTGGGGGC CGTGGCTTGT GCCATGGCTC
501 TGCTGACCCA ACAAACAGAG CTGCAGAGCC TCAGGAGAGA GGTGAGCCGG
551 CTGCAGGGGA CAGGAGGCCC CTCCCAGAAT GGGGAAGGGT ATCCCTGGCA
601 GAGTCTCCCG GAGCAGAGTT CCGATGCCCT GGAAGCCTGG GAGAGTGGGG
651 AGAGATCCCG GAAAAGGAGA GCAGTGCTCA CCCAAAAACA GAAGAAGCAG
701 CACTCTGTCC TGCACCTGGT TCCCATTAAC GCCACCTCCA AGGATGACTC
751 CGATGTGACA GAGGTGATGT GGCAACCAGC TCTTAGGCGT GGGAGAGGCC
801 TACAGGCCCA AGGATATGGT GTCCGAATCC AGGATGCTGG AGTTTATCTG
851 CTGTATAGCC AGGTCCTGTT TCAAGACGTG ACTTTCACCA TGGGTCAGGT
901 GGTGTCTCGA GAAGGCCAAG GAAGGCAGGA GACTCTATTC CGATGTATAA
951 GAAGTATGCC CTCCCACCCG GACCGGGCCT ACAACAGCTG CTATAGCGCA
1001 GGTGTCTTCC ATTTACACCA AGGGGATATT CTGAGTGTCA TAATTCCCCG
1051 GGCAAGGGCG AAACTTAACC TCTCTCCACA TGGAACCTTC CTGGGGTTTG
1101 TGAAACTGTG ATTGTGTTAT AAAAAGTGGC TCCCAGCTTG GAAGACCAGG
1151 GTGGGTACAT ACTGGAGACA GCCAAGAGCT GAGTATATAA AGGAGAGGGA
1201 ATGTGCAGGA ACAGAGGCGT CTTCTGGGT TTGGCTCCCC GTTCCTCACT
1251 TTTCCCTTTT CATTCCCACC CCCTAGACTT TGATTTTACG GATATCTTGC
1301 TTCTGTTCCC CATGGAGCTC CGAATTCCTG CGTGTGTGTA GATGAGGGGC
1351 GGGGGACGGG CGCCAGGCAT TGTTTCAGAC TGGTCGGGGC CCACTGGAAG
1401 CATCCAGAAC AGCACCACCA TCTAACGGCC GCTCGAGGGA AGCACCCGGC
1451 GGTTTGGGCG AAGTC

```

The proposed transmembrane domains are boxed

human G70 protein sequence (SEQ ID NO 2)

```

1  MPASSPFLLA PKGPPGNMGG FVREPALSVA LWLSWGAALG AVACAMALLT
51  QQTELQSLRR EVSRLQGTGG PSQNGEGYPW QSLPEQSSDA LEAWESGERS
101 RKRRAVLTQK QKKQHSVLHL VPINATSKDD SDVTEVMWQP ALRRGRGLQA
151 QGYGVRIQDA GVYLLYSQVL FQDVTFTMGQ VVSREGQGRQ ETLFRCIRSM
201 PSHPDRAVNS CYSAGVFHLH QGDILSVIIP RARAKLNLSP HGTFLGFVKL

```

Figure 2A

Sequence of mouse G70 (SEQ ID NOS: 3 and 4)

Mouse G70 (SEQ ID NO 3)

```
1  CATGCCGAGT GCTTTGTGTG TGTTACCTGC TCTAAGAAGC TGGCTGGGCA
51  GCGTTTCACC GCTGTGGAGG ACCAGTATTA CTGCGTGGAT TGCTACAAGA
101 ACTTTGTGGC CAAGAAGTGT GCTGGATGCA AGAACCCCAT CACTGGGTTT
151 GGTAAGGCT CCAGTGTGGT GGCCTATGAA GGACAATCCT GGCACGACTA
201 CTGCTTCCAC TGCAAAAAAT GCTCCGTGAA TCTGGCCAAC AAGCGCTTTG
251 TATTTCATAA TGAGCAGGTG TATTGCCCTG ACTGTGCCAA AAAGCTGTAA
301 CTTGACGGCT GCCCTGTCCT TCCTAGATAA TGGCACCAA TTCTCCTGAG
351 GCTAGGGGGG AAGGAGTGTC AGAGTGTCAC TAGCTCGACC CTGGGGACAA
401 GGGGGACTAA TAGTACCCTA GCTTGATTTC TTCCTATTCT CAAGTTCCTT
451 TTTATTTCTC CTTGCGTAA CCCGCTCTTC CCTTCTGTGC CTTTGCCTGT
501 ATTCCACCC TCCCTGCTAC CTCTTGGCCA CCTCACTTCT GAGACCACAG
551 CTGTTGGCAG GGTCCCTAGC TCATGCCAGC CTCATCTCCA GGCCACATGG
601 GGGGCTCAGT CAGAGAGCCA GCCCTTTCGG TTGCTCTTTG GTTGAGTTGG
651 GGGGCAGTTC TGGGGGCTGT GACTTGTGCT GTCGCACTAC TGATCCAACA
701 GACAGAGCTG CAAAGCCTAA GGCGGGAGGT GAGCCGGCTG CAGCGGAGTG
751 GAGGGCCTTC CCAGAAGCAG GGAGAGCGCC CATGGCAGAG CCTCTGGGAG
801 CAGAGTCCTG ATGTCCTGGA AGCCTGGAAG GATGGGGCGA AATCTCGGAG
851 AAGGAGAGCA GTACTACCC AGAAGCACAA GAAGAAGCAC TCAGTCCTGC
901 ATCTTGTTCC AGTTAACATT ACCTCCAAGG ACTCTGACGT GACAGAGGTG
951 ATGTGGCAAC CAGTACTTAG GCGTGGGAGA GGCCTGGAGG CCCAGGGAGA
1001 CATTGTACGA GTCTGGGACA CTGGAATTTA TCTGCTCTAT AGTCAGGTCC
1051 TGTTCATGA TGTGACTTTC ACAATGGGTC AGGTGGTATC TCGGGAAGGA
1101 CAAGGGAGAA GAGAACTCT ATTCCGATGT ATCAGAAGTA TGCCTTCTGA
1151 TCCTGACCGT GCCTACAATA GCTGCTACAG TGCAGGTGTC TTTCATTTAC
1201 ATCAAGGGGA TATTATCACT GTCAAAATTC CACGGGCAAA CGCAAAACTT
1251 AGCCTTTCCT CGCATGGAAC ATTCTGGGG TTTGTGAAAC TATGATTGTT
1301 ATAAAGGGGG TGGGGATTTC CCATTCCAAA AACTGGCTAG ACAAAGGACA
1351 AGGAACGGTC AAGAACAGCT CTCCATGGCT TTGCCTTGAC TGTGTTTCCT
1401 CCCTTTGCCT TTCCCGCTCC CACTATCTGG GCTTTGACTC CATGGATATT
1451 AAAAAAGTAG AATATTTTGT GTTTATCTCC CAAAAA
```

Figure 2B

Mouse G70 Length: 241 (SEQ ID NO 4)

```
1  MPASSPGHMG GSVREPALSV ALWLSWGAVL GAVTCAVALL IQQTELQSLR
51  REVSRLQMSG GPSQKQGERP WQSLWEQSPD VLEAWKDGA SRRRAVLTQ
101 KHKKKHSLVH LVPVNITSD SDVTEVMWQP VLRRGRGLEA QGDIVRVWD
151 GIYLLYSQVL FHDVTFTMGQ VVSREGQGR ETLFRCIRSM PSDPDRAINS
201 CYSAGVFHLH QGDIITVKIP RANAKLSLSP HGTFLGFVKL *
```

G-70 FLAG des92 (smuG70) Strain #4081 (SEQ ID NO 19):

```
MDYKDDDDKKHKKKHSLVHLVLPVNITSDSDVTEVMWQPVLRGRGLEAQGDIVRVW
DTGIYLLYSQVL FHDVTFTMGQVVSREGQGR ETLFRCIRSMPSDPDRAYNSCYSAG
VFHLHQGDIITVKIPRANAKLSLSPHGTFLGFVKL*
```

Figure 3
Alignm. of human and mouse G70

```
mouse: 1  MPASS-----PGHMGGSVREPALSVLWLSWGAVLGAVTCAVALLIQQTELOSLRR 51
          MPASS          PG+MGG VREPALSVLWLSWGA LGAV CA+ALL QQTELOSLRR
Human: 1  MPASSPFL LAPKGPPGNMGGPVREPALSVLWLSWGAALGAVACAMALLTQQTELOSLRR 60

mouse: 52 EVSRLQ RSGGPSQKQGERPWQSLWEQSPDVLEAWKDGA KSRRRAVLTQKHKKKH SVLHL 111
          EVSRLQ +GGPSQ      PWQSL EQS D LEAW+ G +SR+RAVLTQK KK+H SVLHL
human: 61 EVSRLQGTGGPSQNGEGYPWQSLPEQSSDALEAWESGERSRKRRAVLTQKQKKQH SVLHL 120

mouse: 112 VPVNITSKD-SDVTEVMWQPVLRRGRGLEAQGDIVRVWDTGIYLLYSQVLFHDVTFTMGQ 170
          VP+N TSKD SDVTEVMWQP LRRGRGL+AQG VR+ D G+YLLYSQVLF DVTFTMGQ
human: 121 VPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQ 180

mouse: 171 VVSREGQGRRETLFRCIRSMPSDPDRAYNSCYSAGVFHLHQGDIITVKIPRANAKLSLSP 230
          VVSREGQGR+ETLFRCIRSMPS PDRAYNSCYSAGVFHLHQGDI++V IPRA AKL+LSP
human: 181 VVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARA KLNLSLSP 240

mouse: 231 HGTFLGFVKL 240
          HGTFLGFVKL
human: 241 HGTFLGFVKL 250
```

Fig. 4A

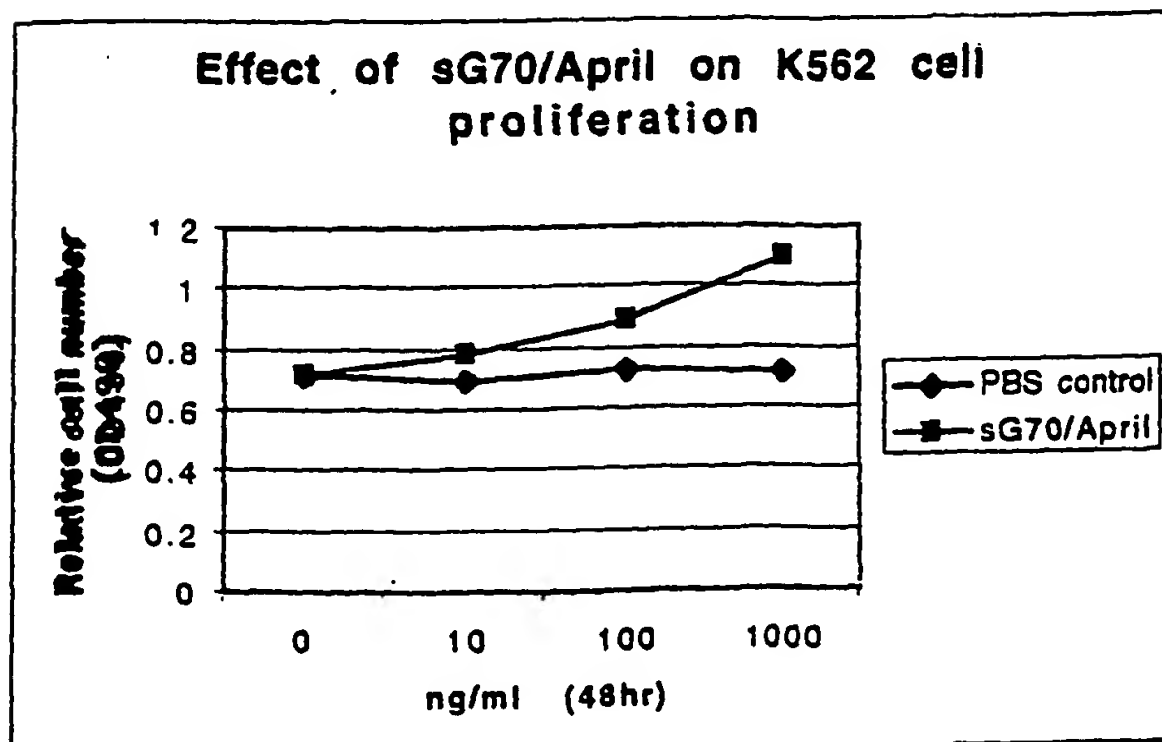
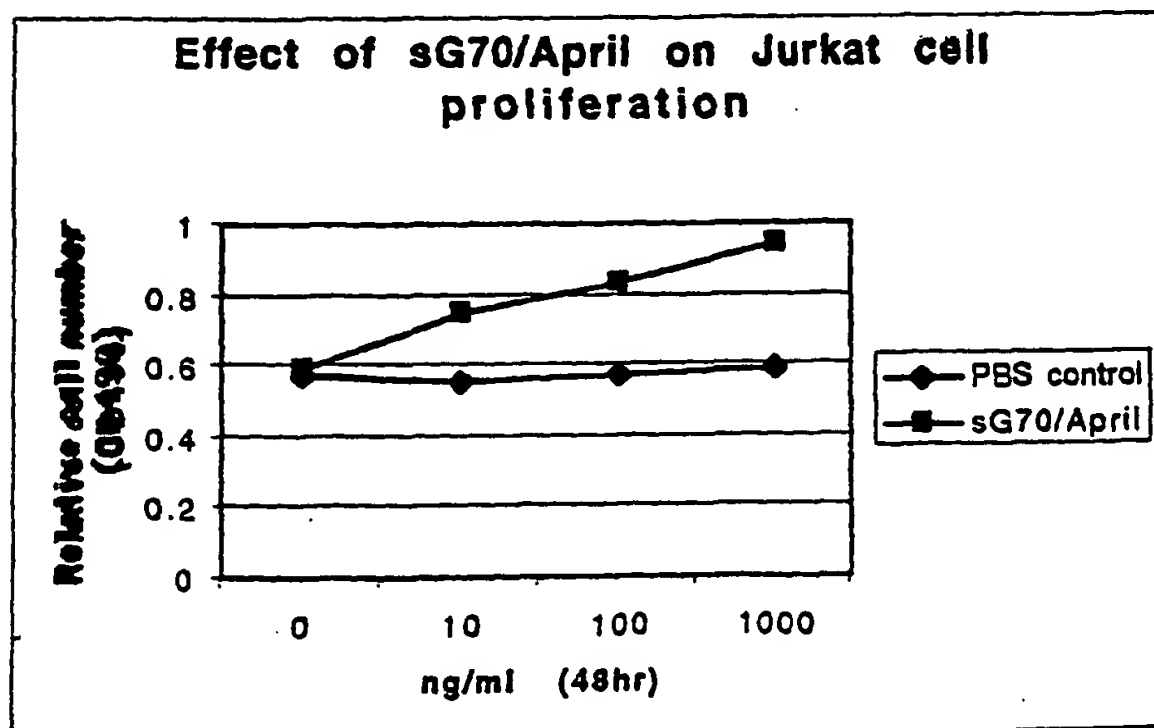
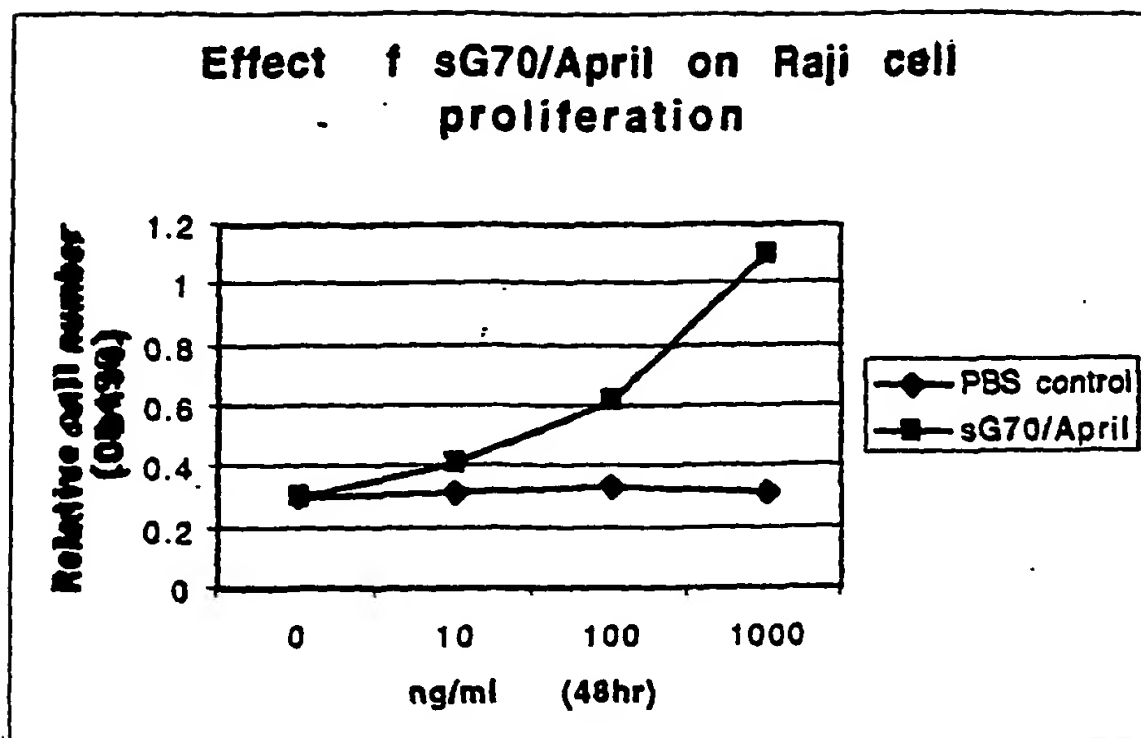


Fig. 4B

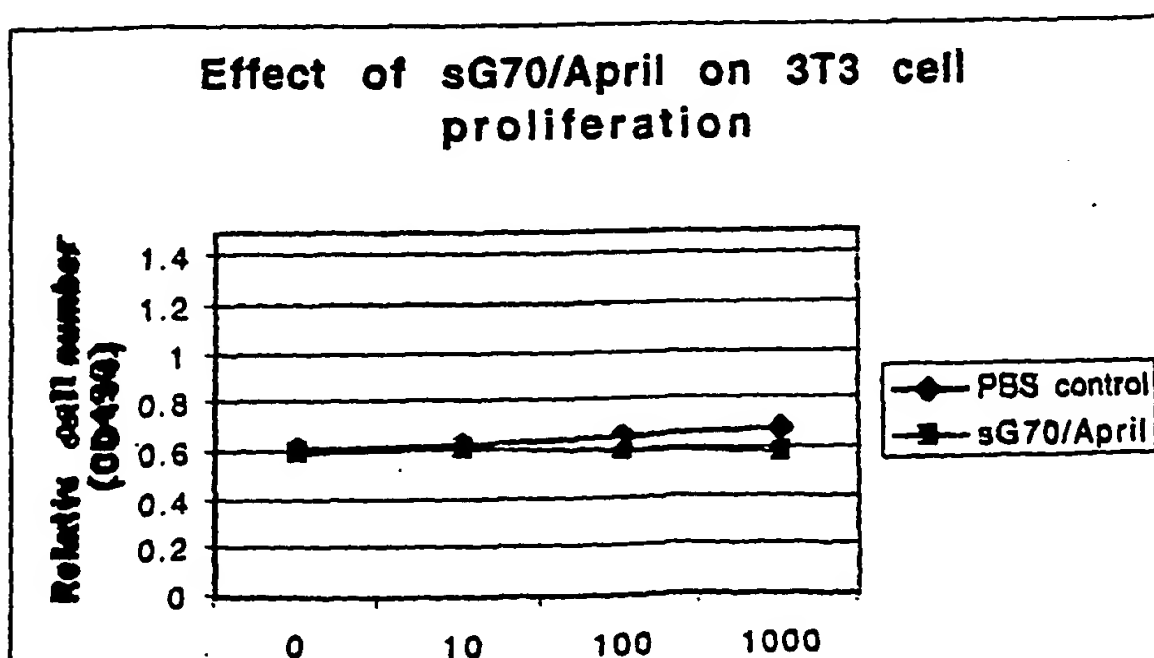
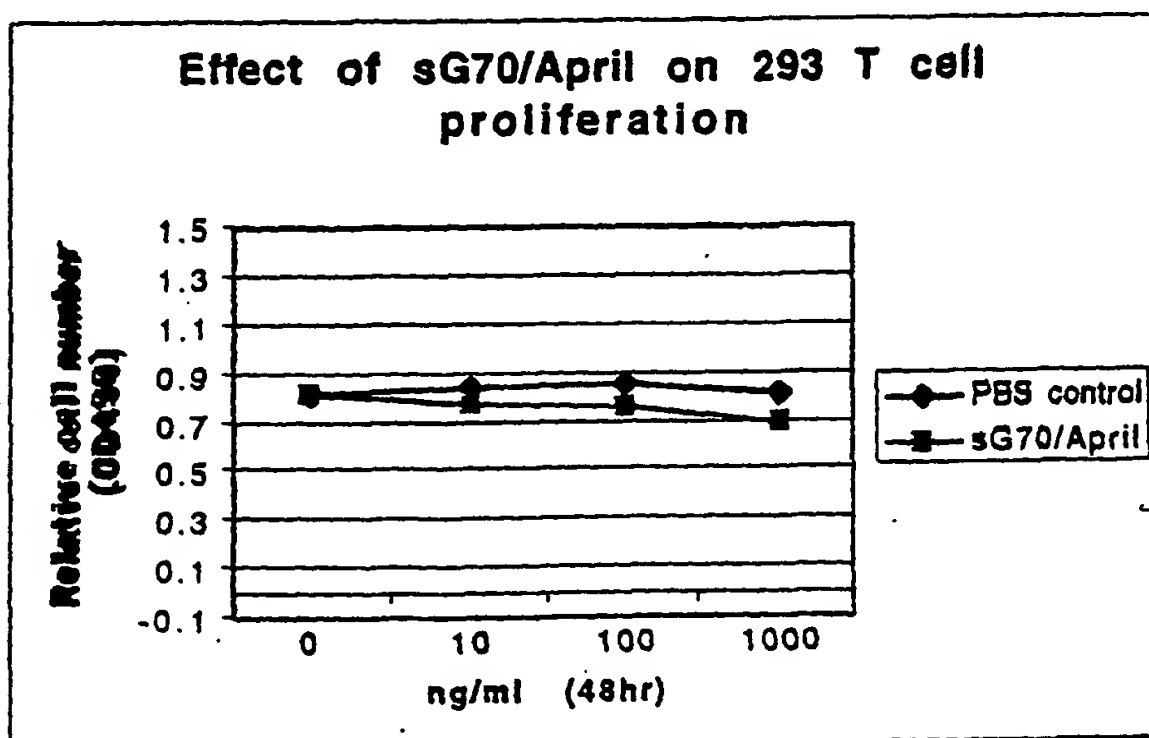
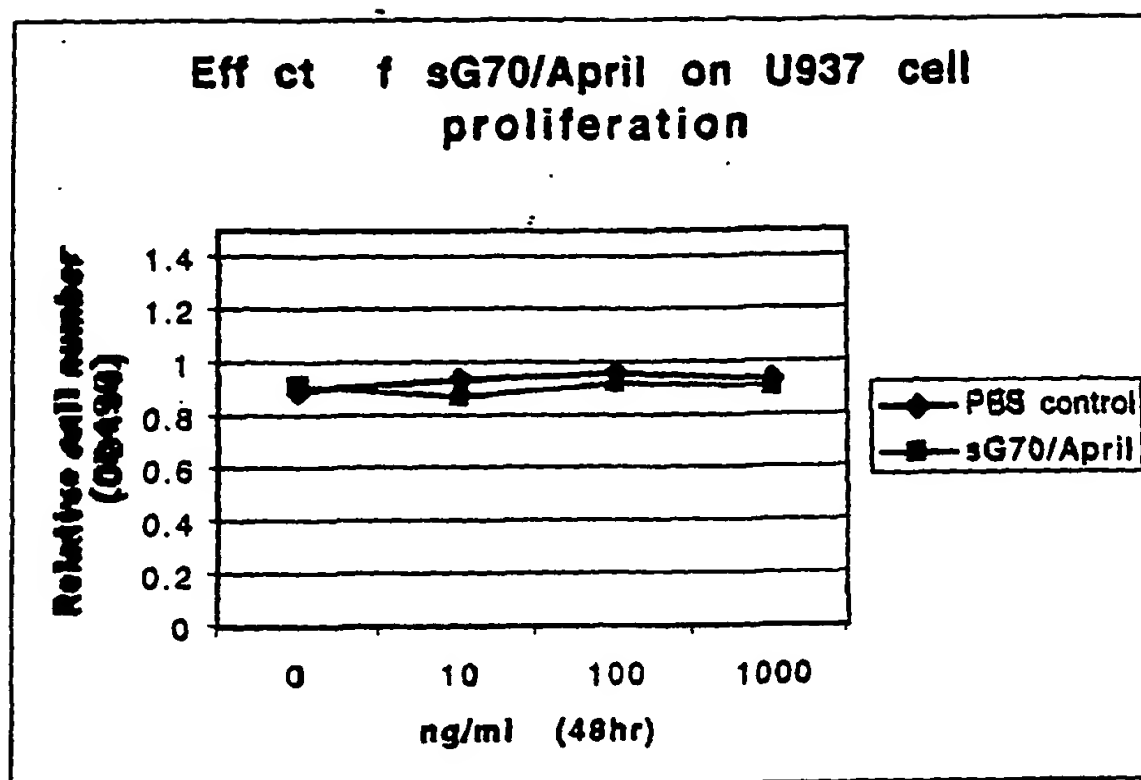
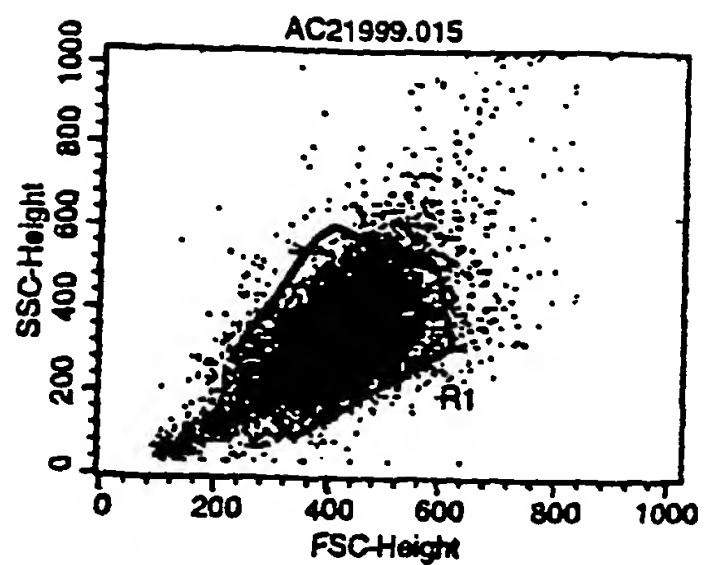


FIGURE 7/48



FACS analysis of G70/April receptor binding

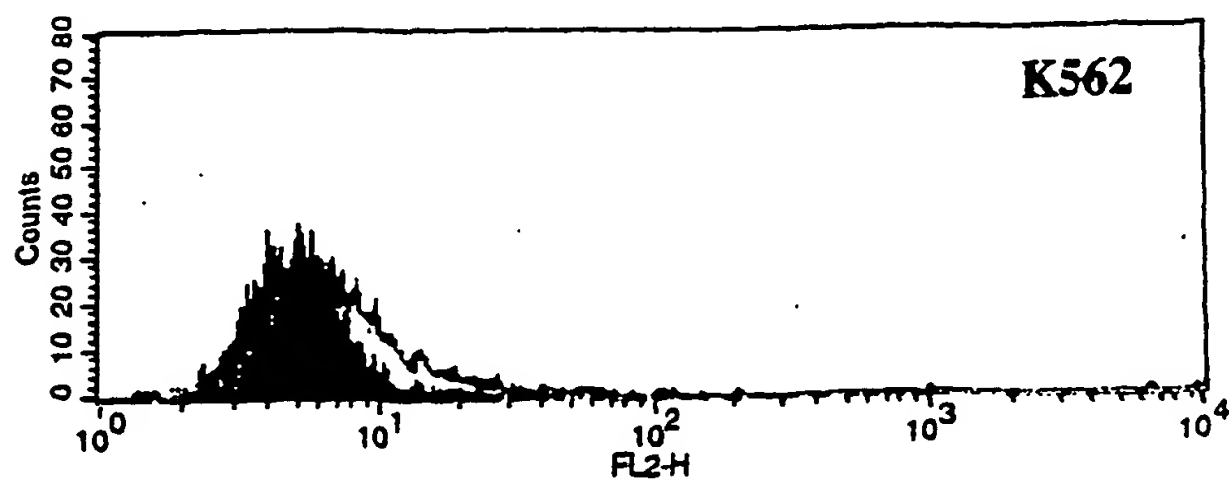
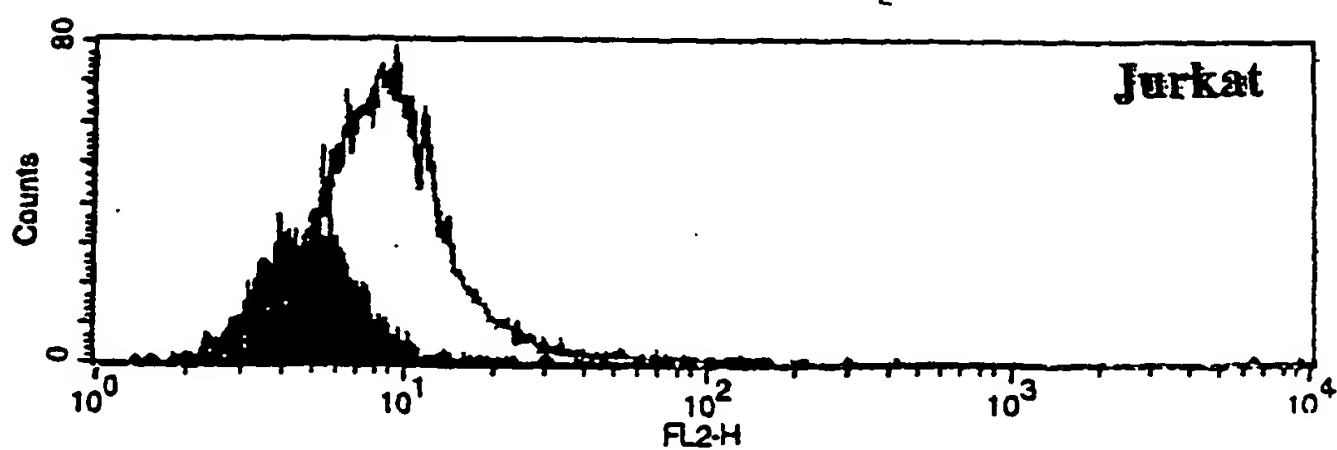
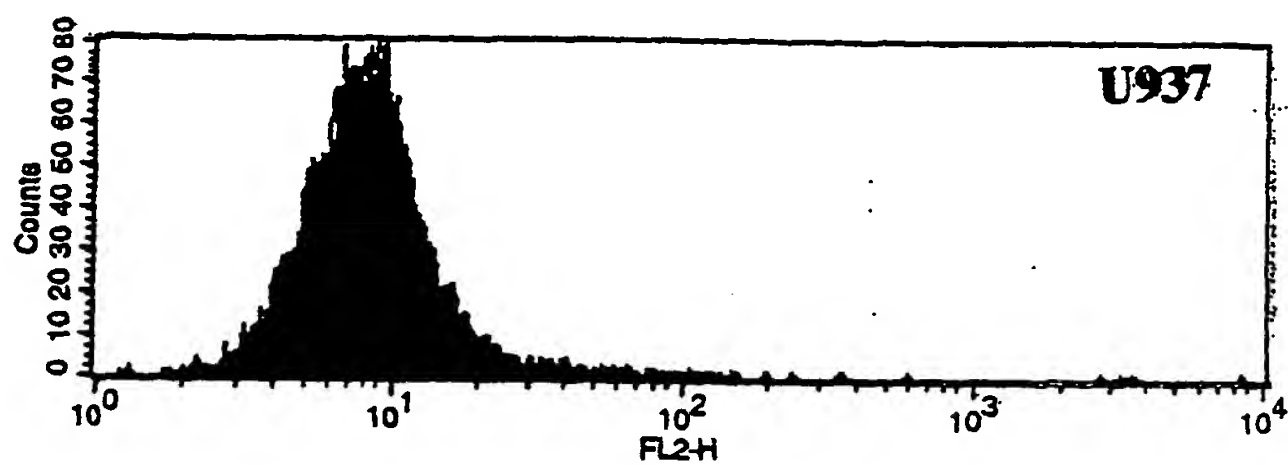


FIGURE 5B

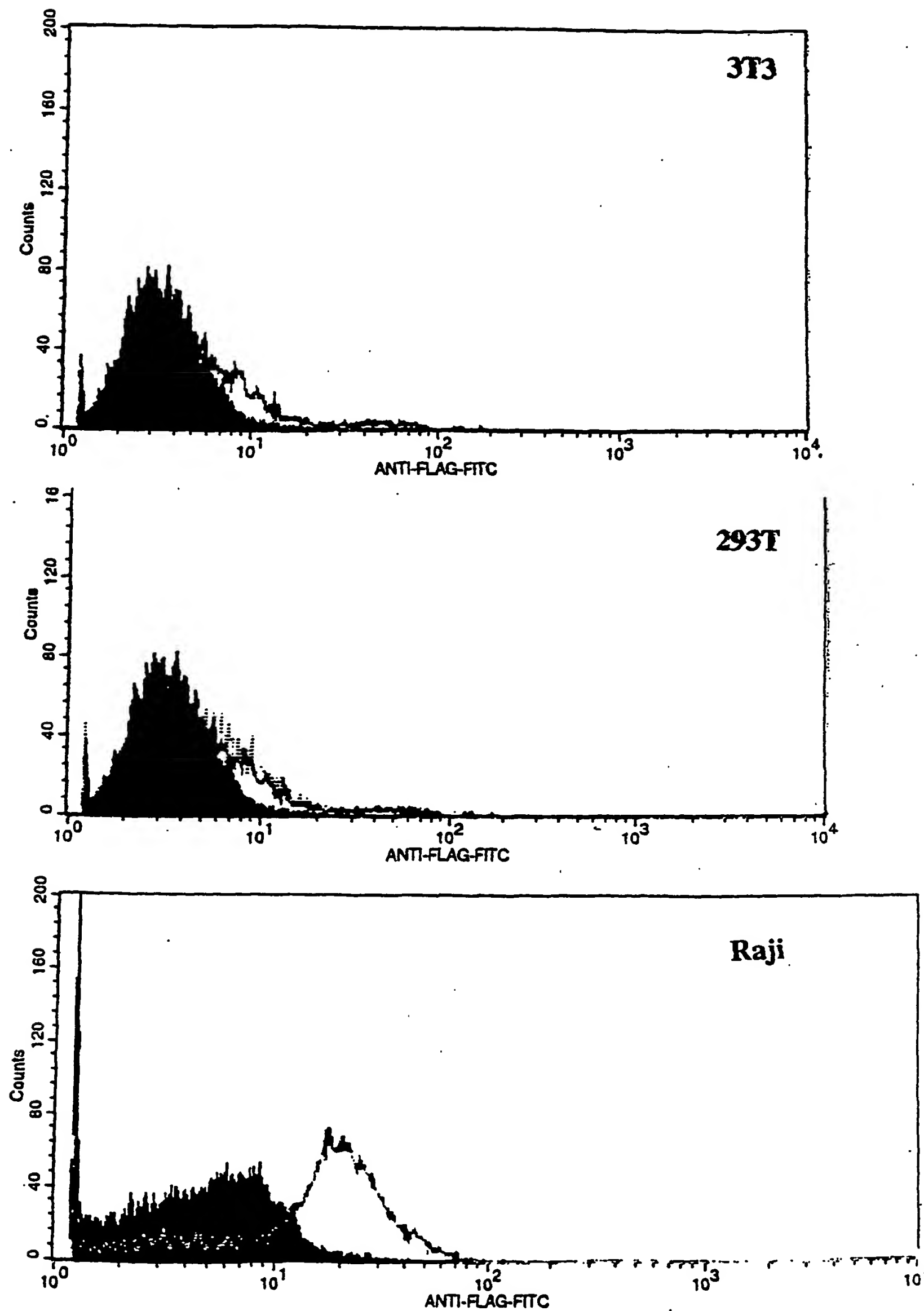


Fig. 6

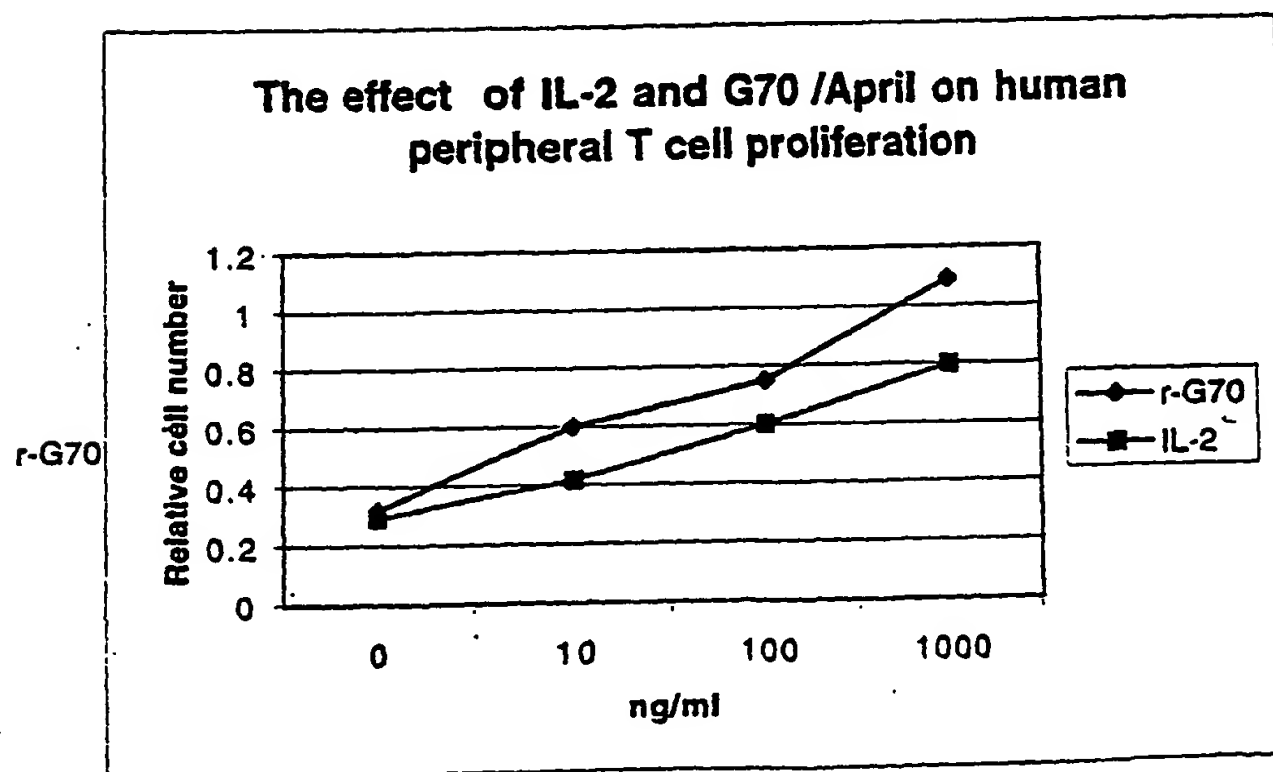
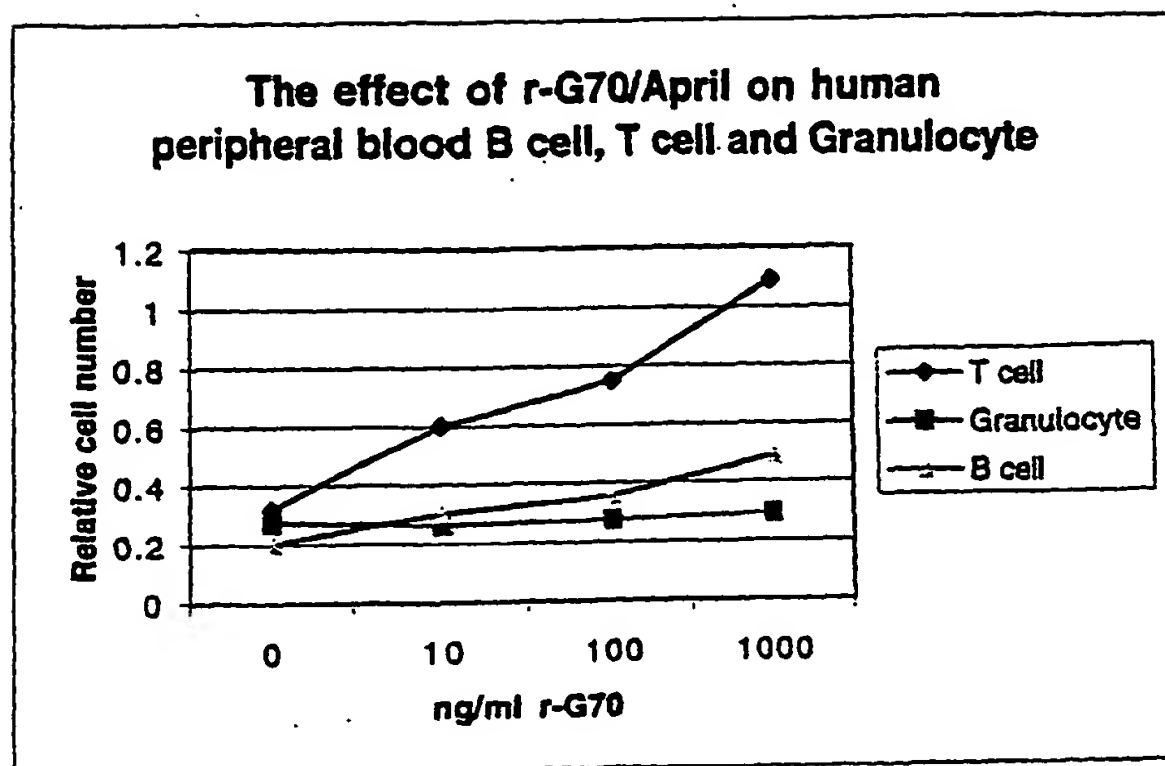


Fig. 7

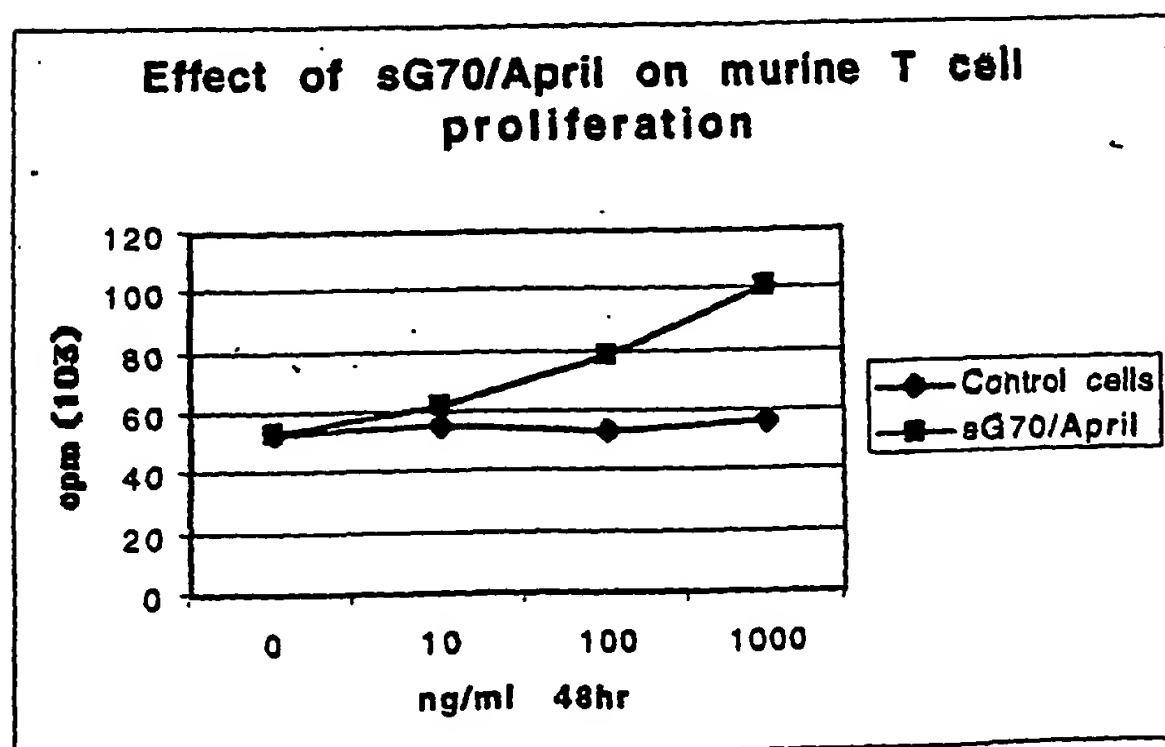
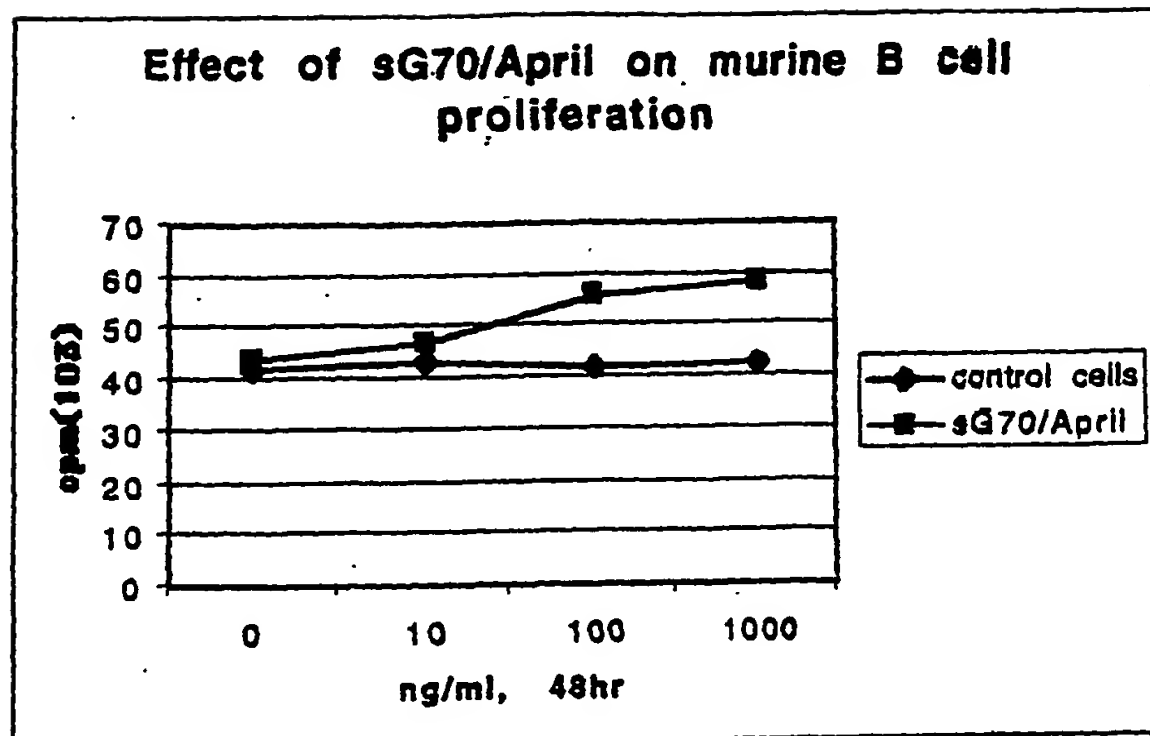


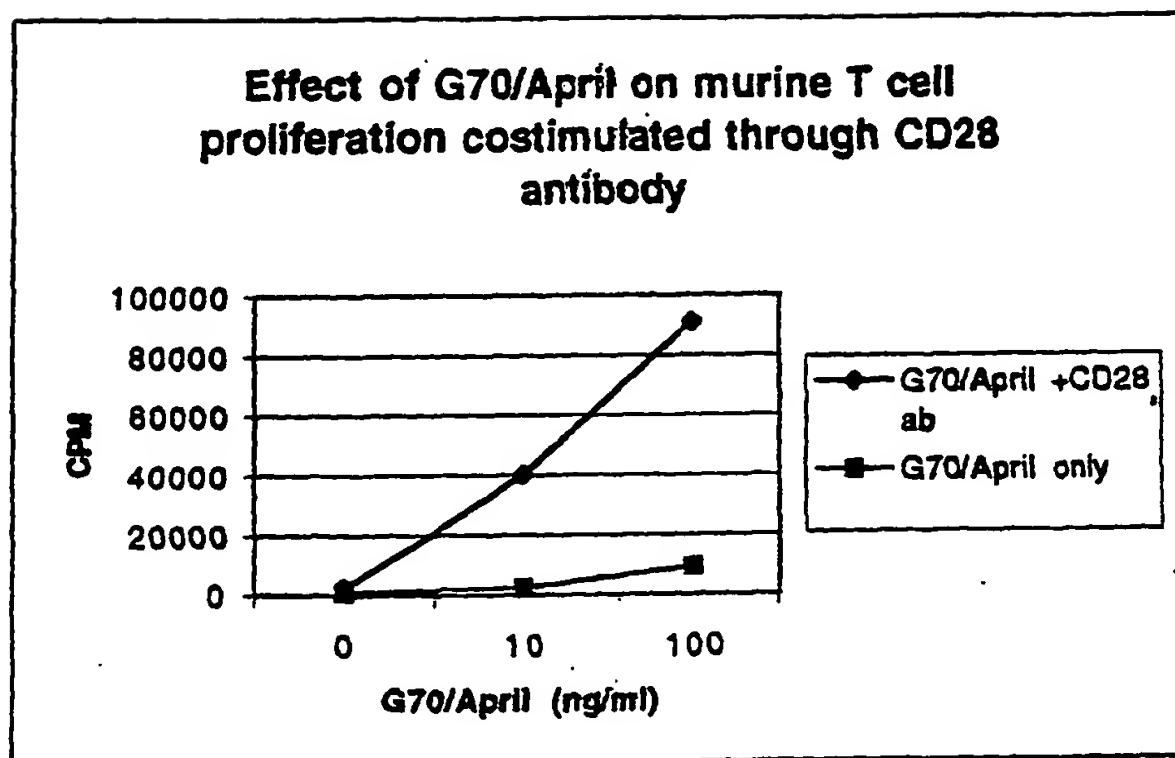
Fig. 8

Fig. 9

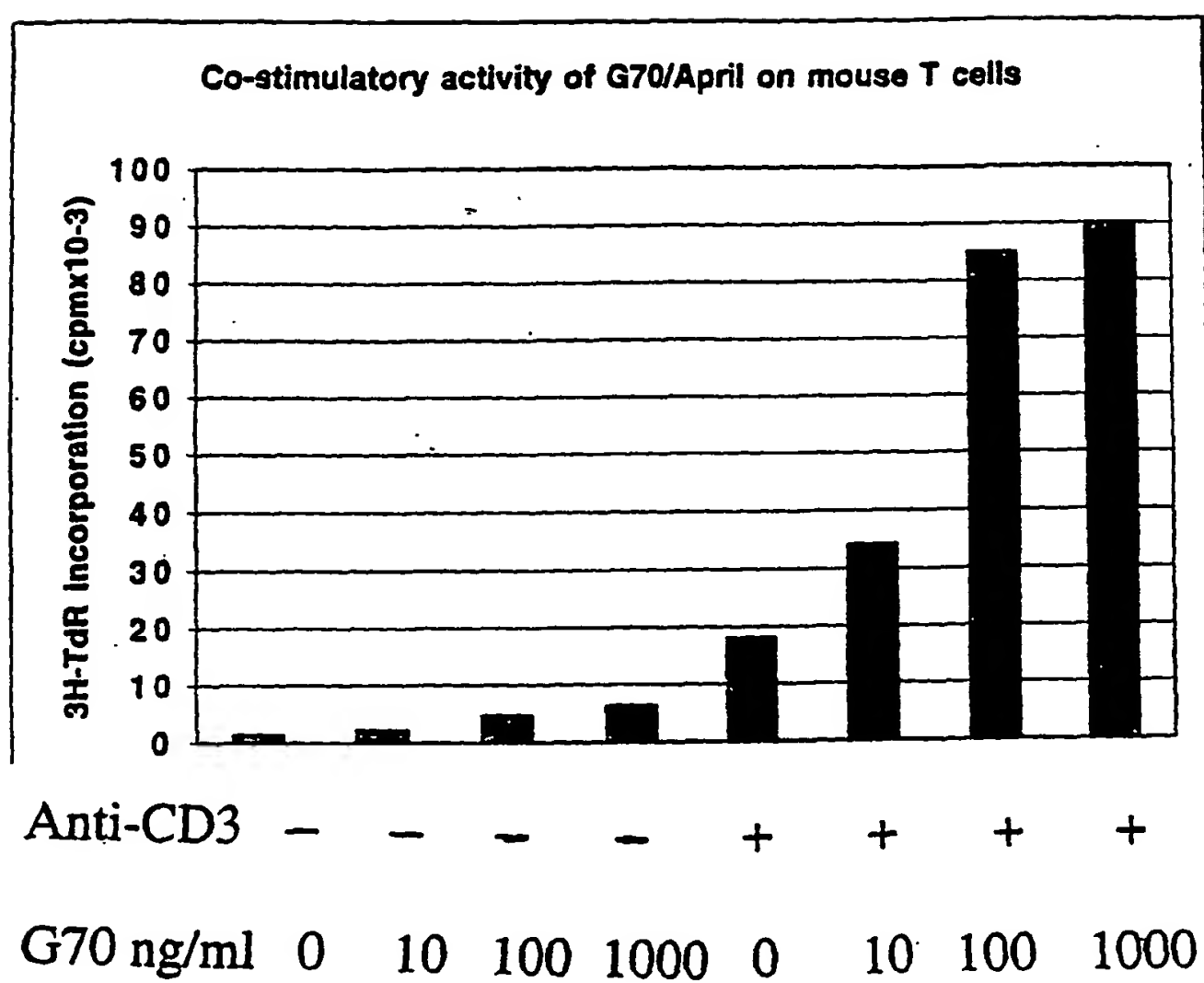


Figure 10A

Human BCMA

Human (SEQ ID NO: 5):

1 MAGQCSQNEY FDSLLHACIP CQLRCSSNTP PLTCQRYCNA
SVTNSVKGTN

51 AILWTCLGLS LIISLAVFVL MFLLRKISSE PLKDEFKNTG
SGLLGMANID

101 LEKSRTGDEI ILPRGLEYTV EECTCEDCIK SKPKVDS DHC
FPLPAMEEGA

151 TILVTTKTND YCKSLPAALS ATEIEKSISA R

Human (SEQ ID NO: 5):

MAGQCSQ NEYFDSLLHA CIPCQLRCSS NTPPLTCQRY CNASVTNSVK
GTNA ILWTCL GLSLIISLAV FVLMFLLRKI SSEPLKDEFK NTGSGLLGMA
NIDLEKSRTG DEILPRGLE YTVEECTCED CIKSKPKVDS DHC FPLPAME
EGATILVTTK TNDYCKSLPA ALSATEIEKS ISAR

hBCMA's extracellular domain (SEQ ID NO: 6):

MAGQCSQ NEYFDSLLHA CIPCQLRCSS NTPPLTCQRY CNASVTNSVK
GTNA

hBCMA's cysteine-rich consensus region (SEQ ID NO: 7):

CSQ NEYFDSLLHA CIPCQLRCSS NTPPLTCQRY C

hBCMA's transmembrane region (SEQ ID NO: 8):

ILWTCL GLSLIISLAV FVLMF

Figure 10B

huBCMA-Fc (SEQ ID NO: 9):

MAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVKGTNA
GGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK*

muBCMA-Fc (SEQ ID NO: 10):

MAQQCFHSEYFDSLLHACKPCHLRCSNPPATCQPYCDPSVTSSVKGSYTG
GGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK*

Figure 11
Alignment of human BCMA amino acid sequence and
murine BCMA amino acid sequence

murine BCMA amino acid sequence Length: 185 (SEQ ID NO: 11):

```
1  MAQQCFHSEY FDSLLHACKP CHLRCSNPPA TCQPYCDPSV TSSVKGTYYTV
51 LWIFLGLTLV LSLALFTISF LLRKMNPEAL KDEPQSPGQL DGSAQLDKAD
101 TELTRIRAGD DRIFPRSLEY TVEECTCEDC VKSKPKGDS HFFPLPAMEE
151 GATILVTTKT GDYGKSSVPT ALQSVGMMEK PTHTR
```

alignment of human BCMA amino acid sequence and murine BCMA amino acid sequence.

```
Query:      4  MAGQCSQNEYFDSLLHACIPCQLRCSNTPPLTCQRYCNASVTNSVKGTNAILWTCLGLS 63
             MA QC +EYFDSLLHAC PC LRCS+ PP TCQ YC+ SVT+SVKGT +LW LGL+
Sbjct:      1  MAQQCFHSEYFDSLLHACKPCHLRCSN--PPATCQPYCDPSVTSSVKGTYYTVLWIFLGLT 58

Query:     64  LIISLAVFVLMFLLRKISSEPLKDEFKNTG----SGLLGMANIDLEKSRTGDEIILPRGL 119
             L++SLA+F + FLLRK++ E LKDE ++ G S L A+ +L + R GD+ I PR L
Sbjct:     59  LVLSLALFTISFLLRKMNPEALKDEPQSPGQLDGSAQLDKADTELTRIRAGDDRIFPRS 118

Query:    120  EYTVVEECTCEDCIKSKPKVDSHCFPLPAMEEGATILVTTKTNDYCKS-LPAAL-SATEI 177
             EYTVVEECTCEDC+KSKPK DSDH FPLPAMEEGATILVTTKT DY KS +P AL S +
Sbjct:    119  EYTVVEECTCEDCVKSKPKGDSHFFPLPAMEEGATILVTTKTGDYGKSSVPTALQSVGM 178

Query:    178  EKSISAR 184
             EK R
Sbjct:    179  EKPTHTR 185
```

Figure 12A

Human TACI

huTACI (SEQ ID NO: 14).

1 MSGLGRSRRG GRSRVDQEER FPQGLWTGVA MRSCPEEQYW DPLLGTSCMSC
51 KTICNHQSQR TCAAFCRSLSCRKEQGKFYD HLLRDCISCA SICGQHPKQC
101 AYFCENKLRS PVNLPPELRR QRSGEVENNS DNSGRYQGLE HRGSEASPAL
151 PGLKLSADQV ALVYSTLGLC LCAVLCCFLV AVACFLKKRG DPCSCQPRSR
201 PRQSPAKSSQ DHAMEAGSPV STSPEPVETC SFCFPECRAP TQESAVTPGT
251 PDPTCAGRWG CHTRTTVLQP CPHIPDSGLG IVCVPAQEGG PGA

MSGLGRSRRGGRSRVDQEERFPQGLWTGVAMRSCPEEQYWDPLLGTSCMSC
KTICNHQSQR TCAAFCRSLSCRKEQGKFYD HLLRDCISCASICGQHPKQC
AYFCENKLRS PVNLPPELRR QRSGEVENNS DNSGRYQGLE HRGSEASPAL
PGLKLSADQV ALVYSTLGLC LCAVLCCFLV AVACFLKKRG DPCSCQPRSR
PRQSPAKSSQ DHAMEAGSPV STSPEPVETC SFCFPECRAP TQESAVTPGT
PDPTCAGRWG CHTRTTVLQP CPHIPDSGLG IVCVPAQEGG PGA

huTACT's extracellular domain (SEQ ID NO: 15):

1 MSGLGRSRRG GRSRVDQEER FPQGLWTGVA MRSCPEEQYW DPLLGTSCMSC
51 KTICNHQSQR TCAAFCRSLSCRKEQGKFYD HLLRDCISCA SICGQHPKQC
101 AYFCENKLRS PVNLPPELRR QRSGEVENNS DNSGRYQGLE HRGSEASPAL
151 PGLKLSADQV ALVYST

Figure 12B

huTACI's cysteine-rich consensus region (SEQ ID NO: 16):

CPEEQYWDPLLGTCTMSCKTICNHQSQR TCAAF C and
CRKEQGKFYDHLLRDCISCASICGQHHPKQCA YFC

transmembrane region (SEQ ID NO: 17):

LGLCLCAVLCCFLVAVACFL

hTACI-Fc (SEQ ID NO: 18):

1 MSGLGRSRRG GRSRVDQEER FPQGLWTGVA MRSCPEEQYW DPLLGTCTMSC

51 KTICNHQSQR TCAAFCRSL S CRKEQGKFYD HLLRDCISCA SICGQHHPKQC

101 AYFCENKLRS PVNLPPELRR QRSGEVENNS DNSGRYQGLE HRGSEASPAL

151 PGLKLSADQV ALVYSGGGGG DKTHTCPPCP APELLGGPSV FLFPPKPKDT

201 LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY

251 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT

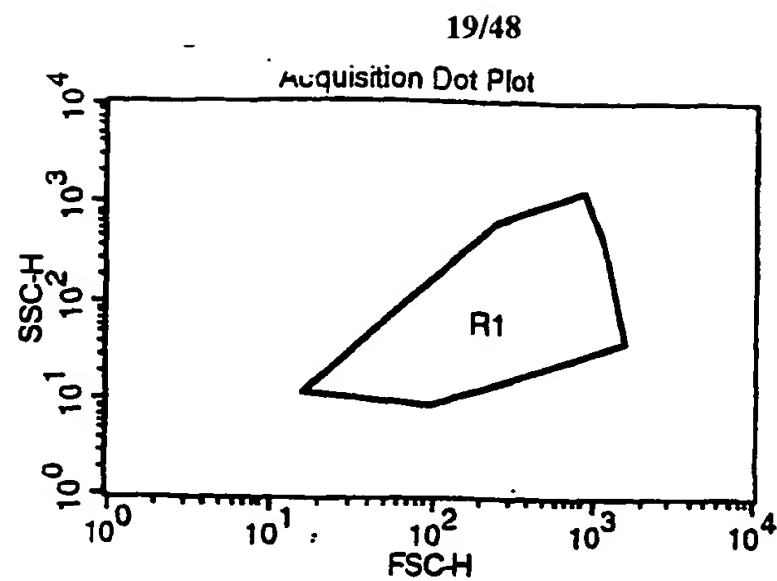
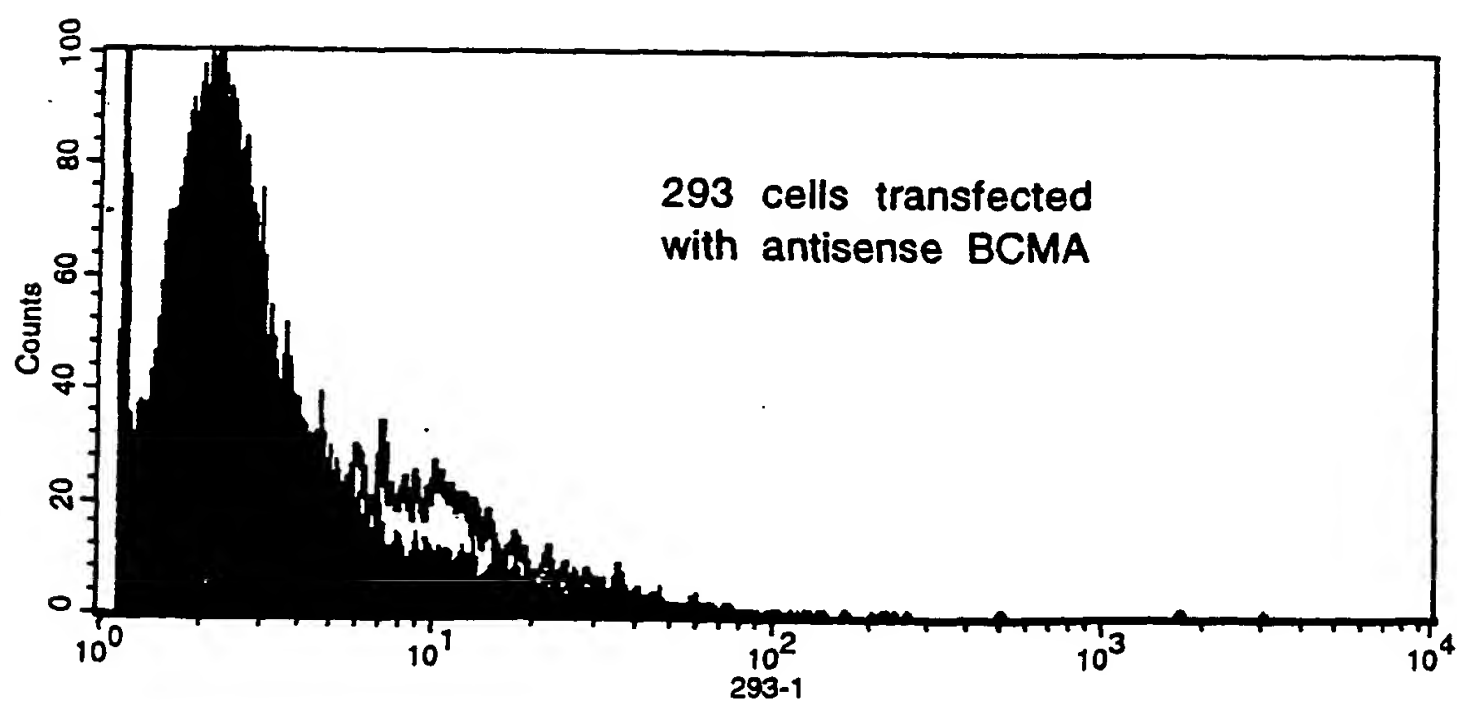
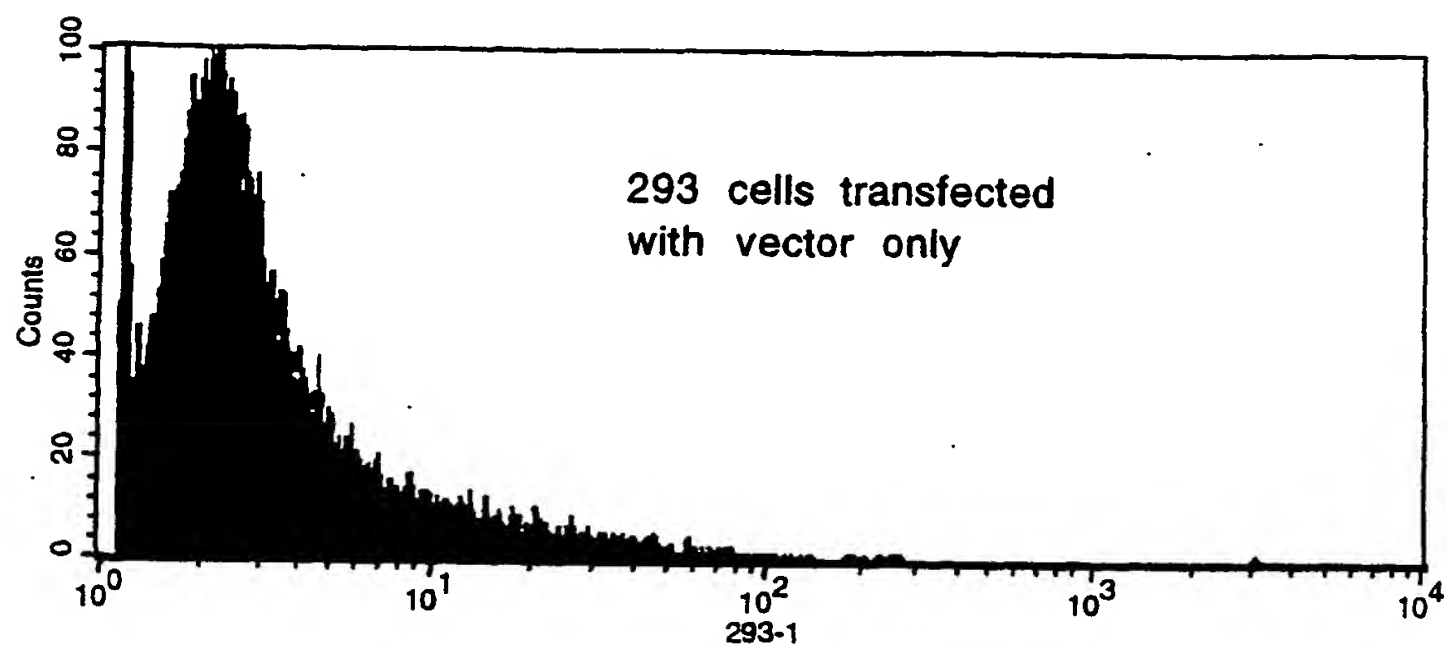
301 LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS

351 DGSFFLYSKL TVDKSRWQQG NVFSCSV MHE ALHNHYTQKS LSLSPGK*

Figure 13**Alignment of cysteine rich extracellular regions of human
TACI and human BCMA.**

```
34 CPEEQYWDPLLGTCTMSCKTICNHQS.QRTCAAFCSRSLSCRKEQGKFDHL 82
   | : : | . | | | . | . | . . | | : | . . | :
8  CSQNEYFDSLHLHACIPQLRCSSNTPPLTCQRYCNASVTNSVKGT..NAI 55

      83 LRDCISCASI 92
      | : . |
56 LWTCLGLSLI 65
```

**Fig.14**

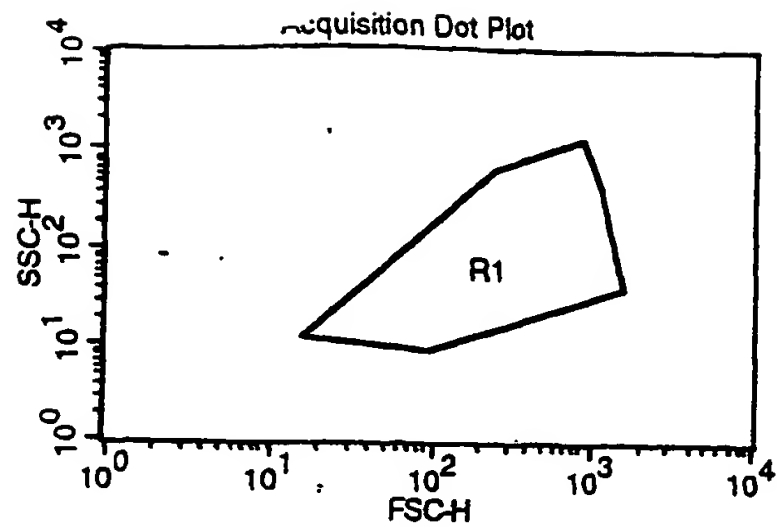
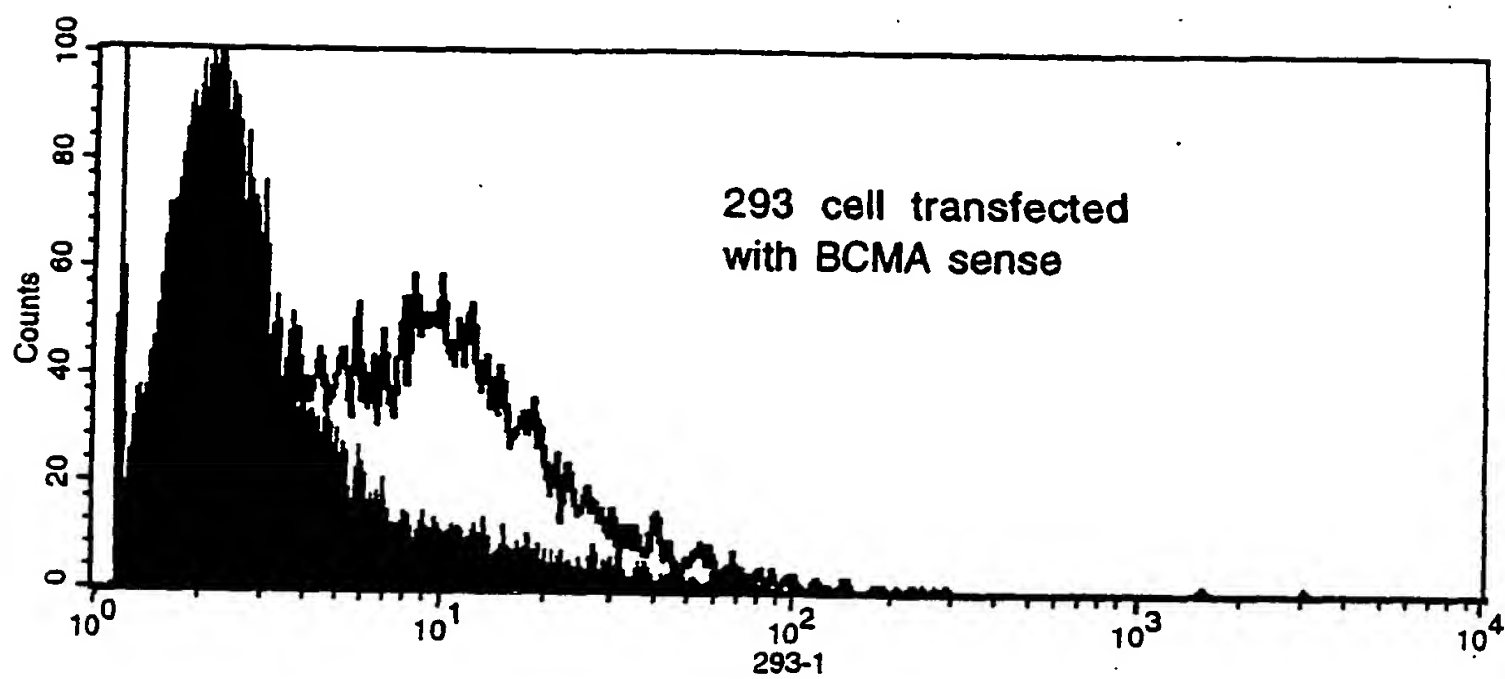


Fig.14



C.

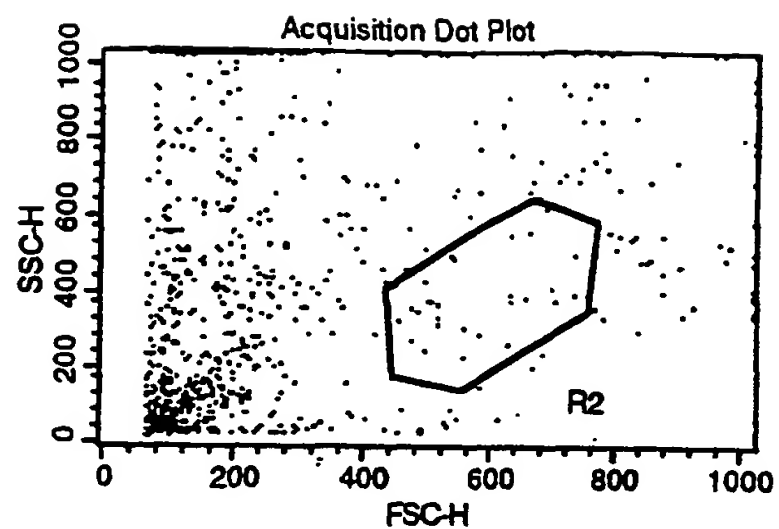
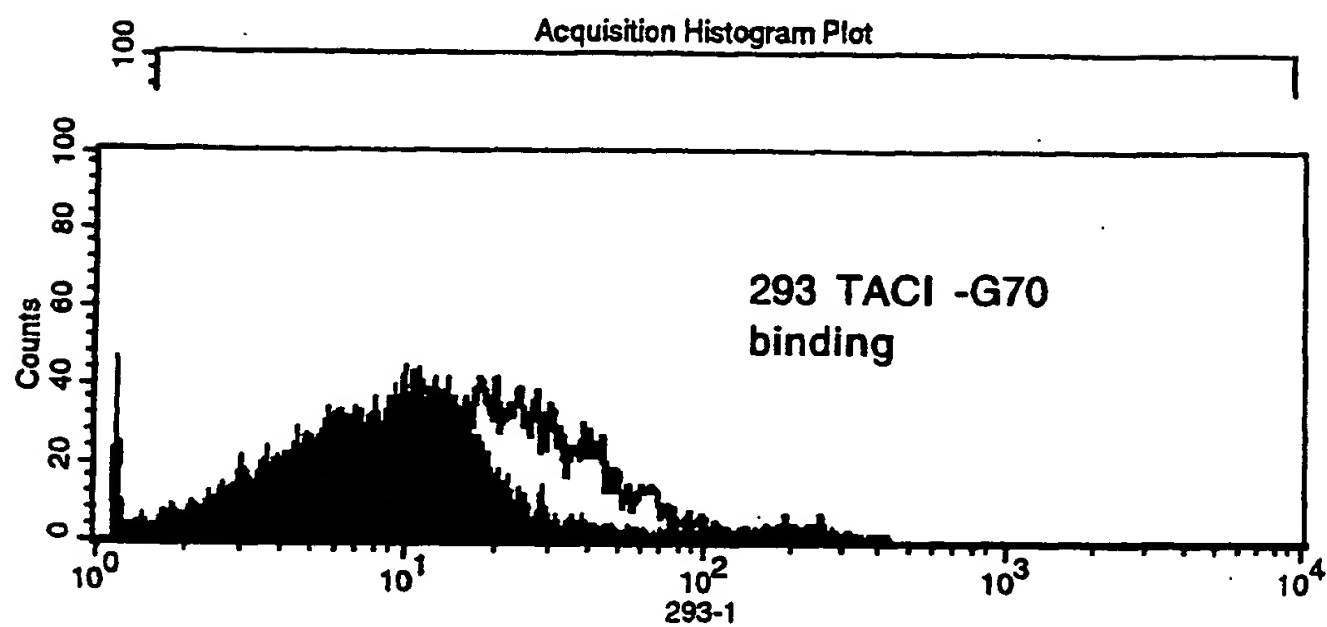
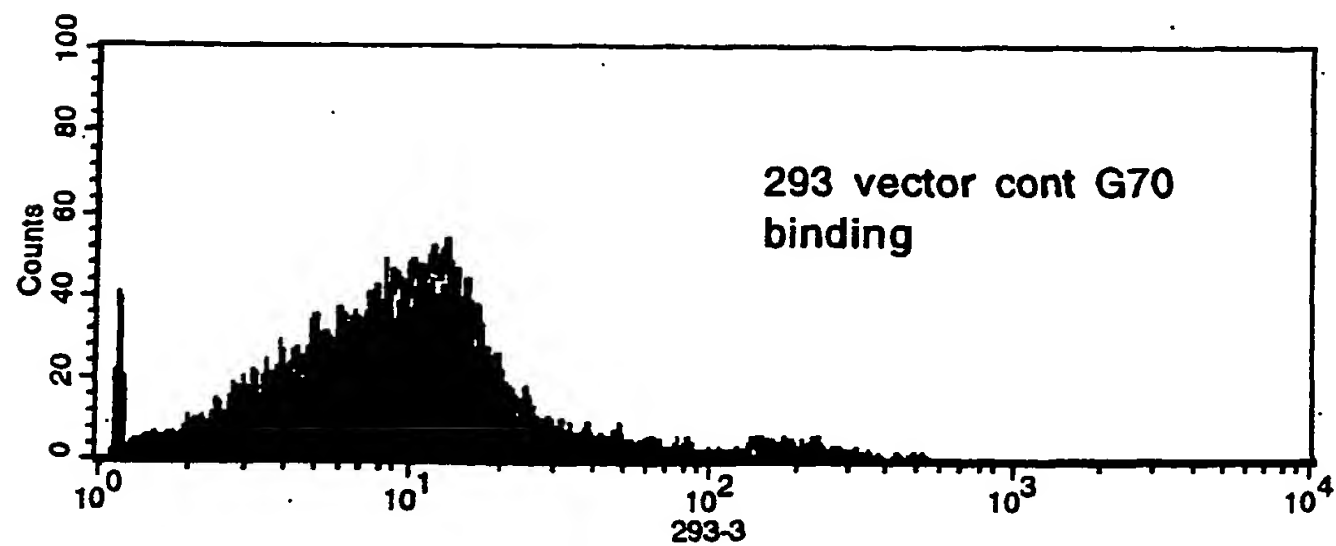


Fig. 15



A.



B.

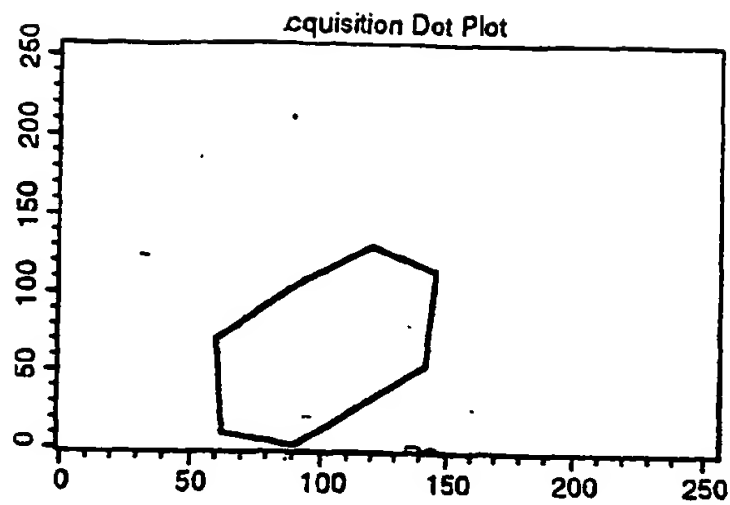
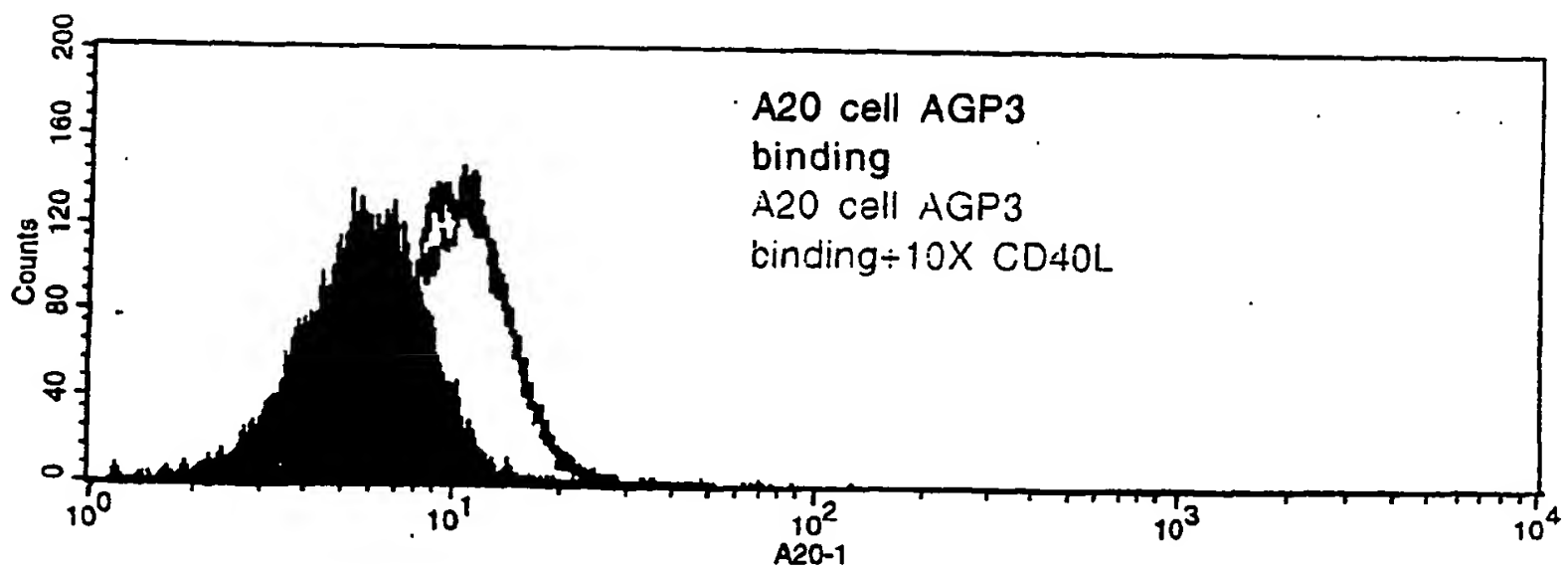
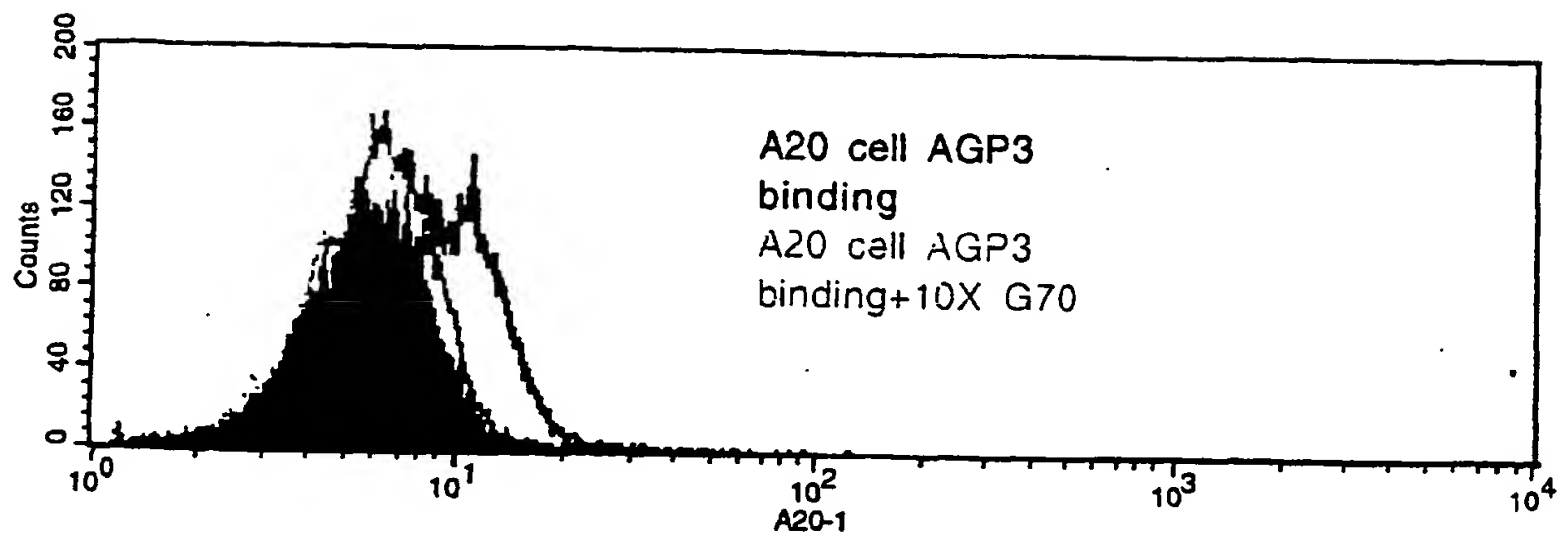
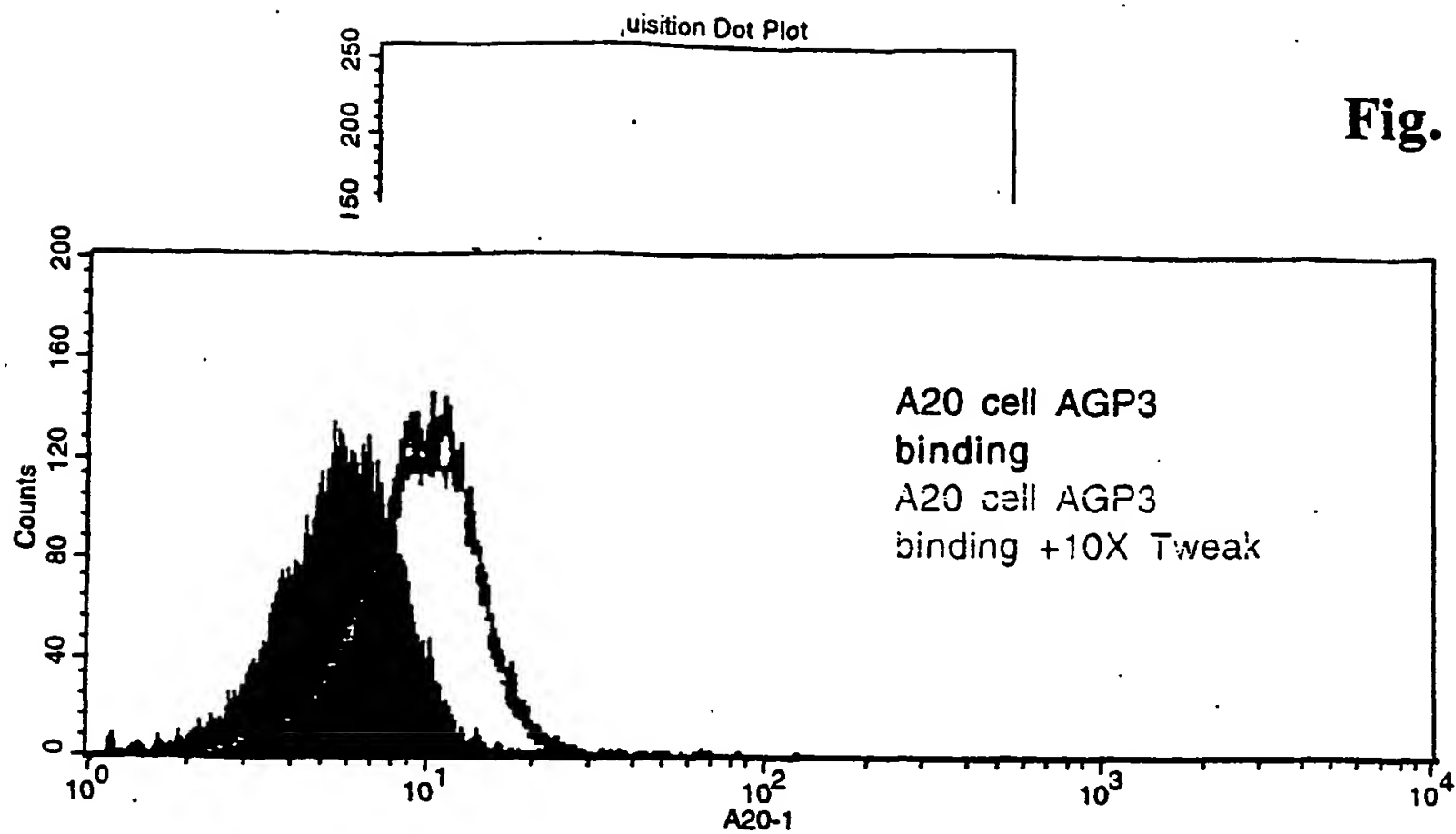


Fig. 16

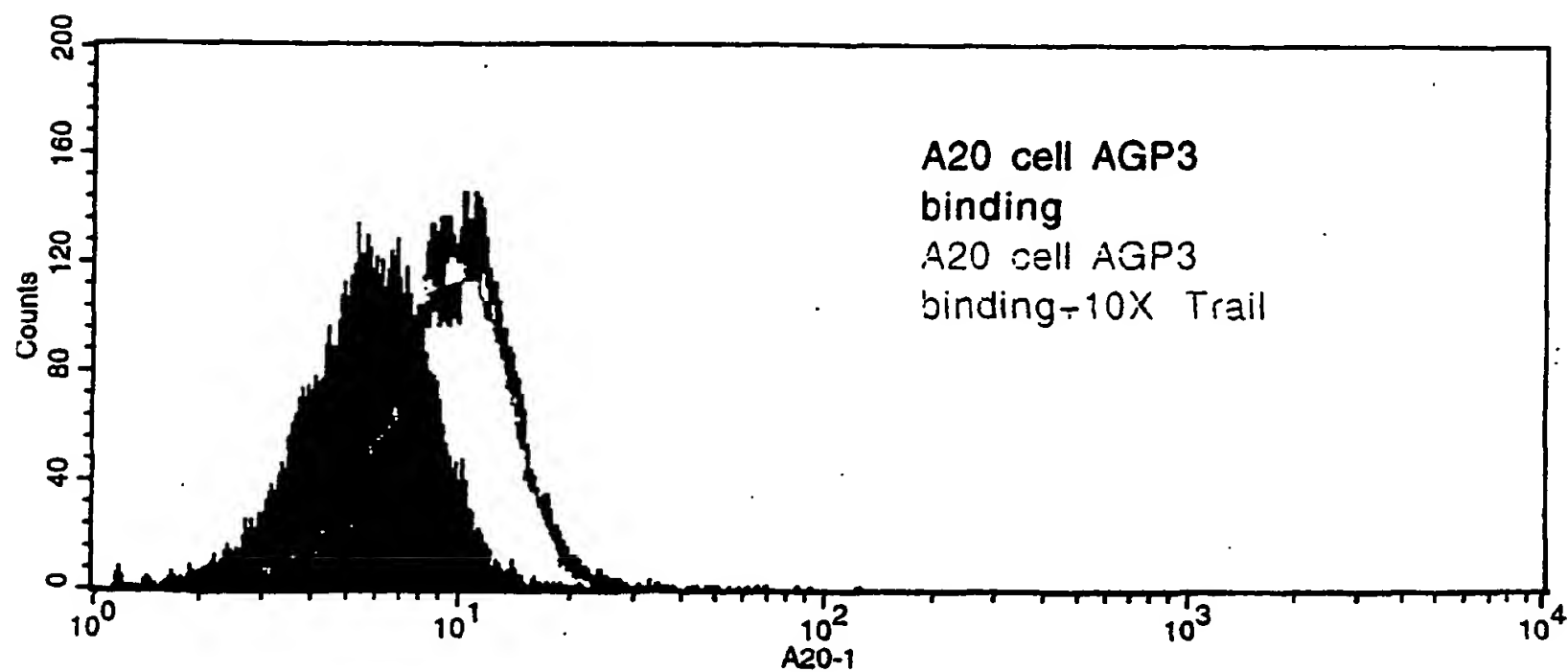


Experiment 4-3-2000

Fig. 16



C.

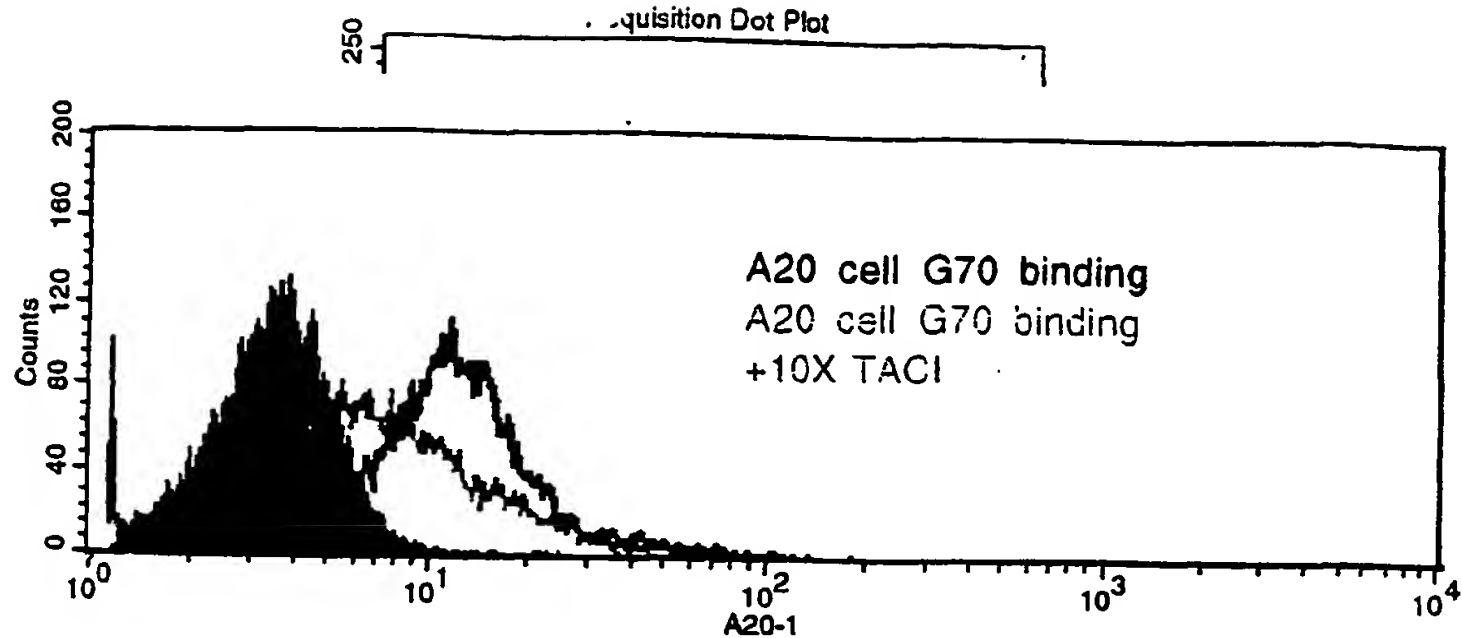


D.

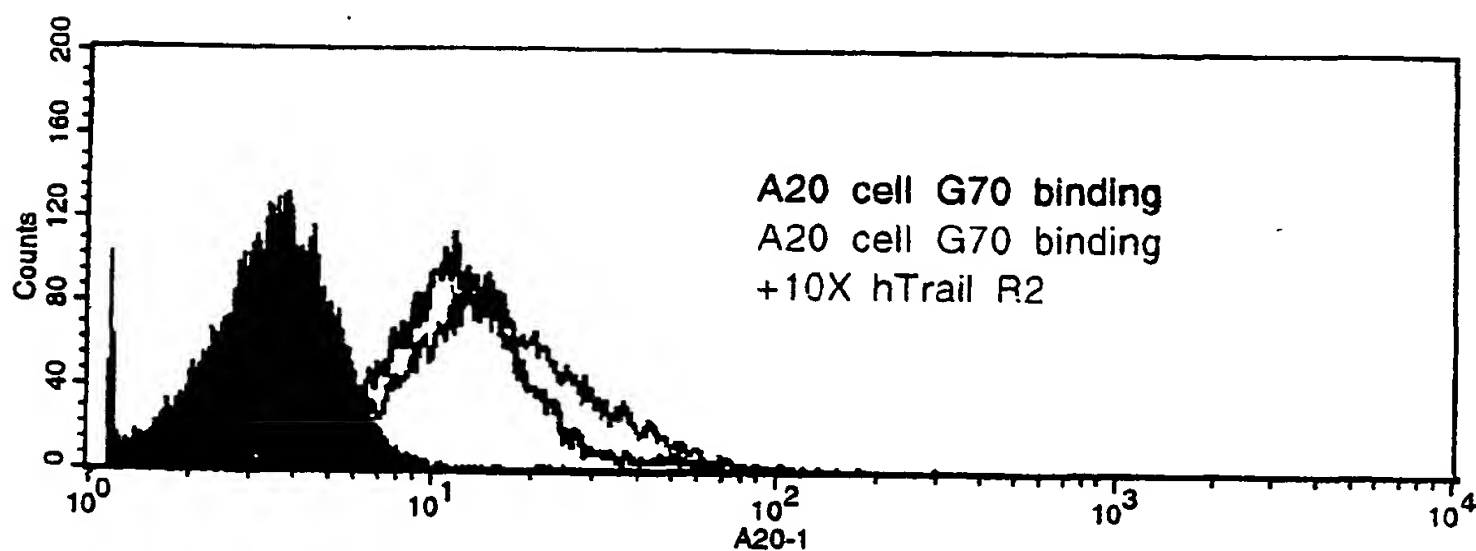
Experiment 4-3-2000

Fig.17

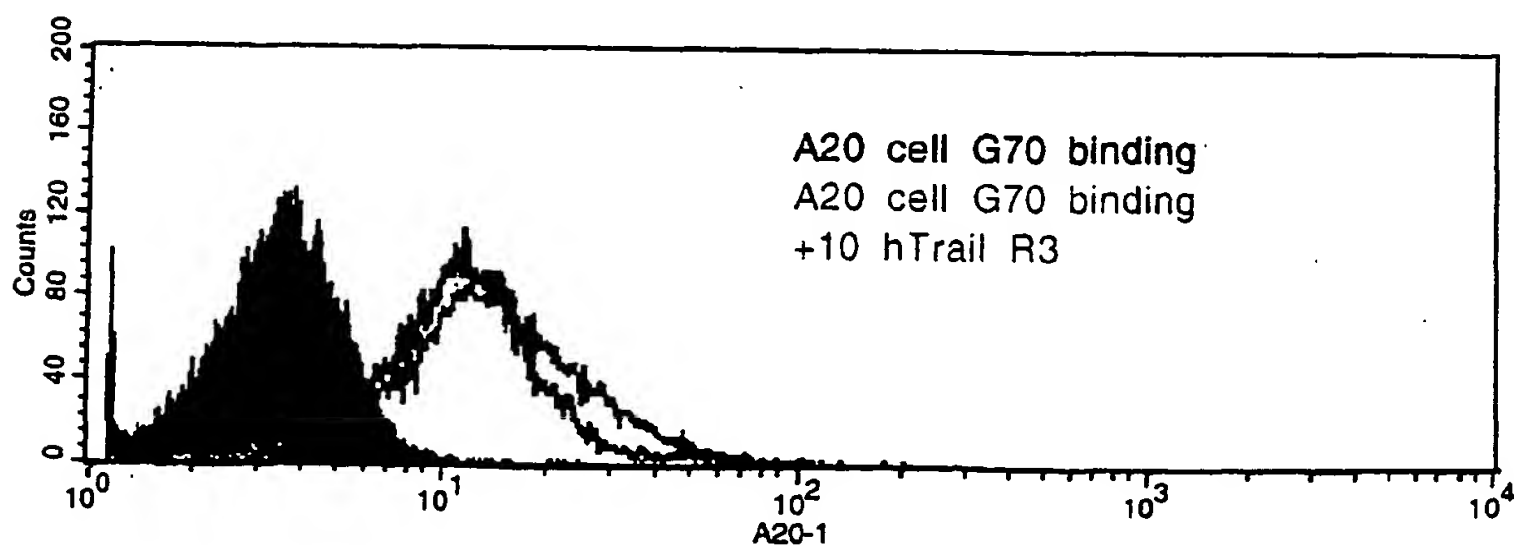
A.



B.

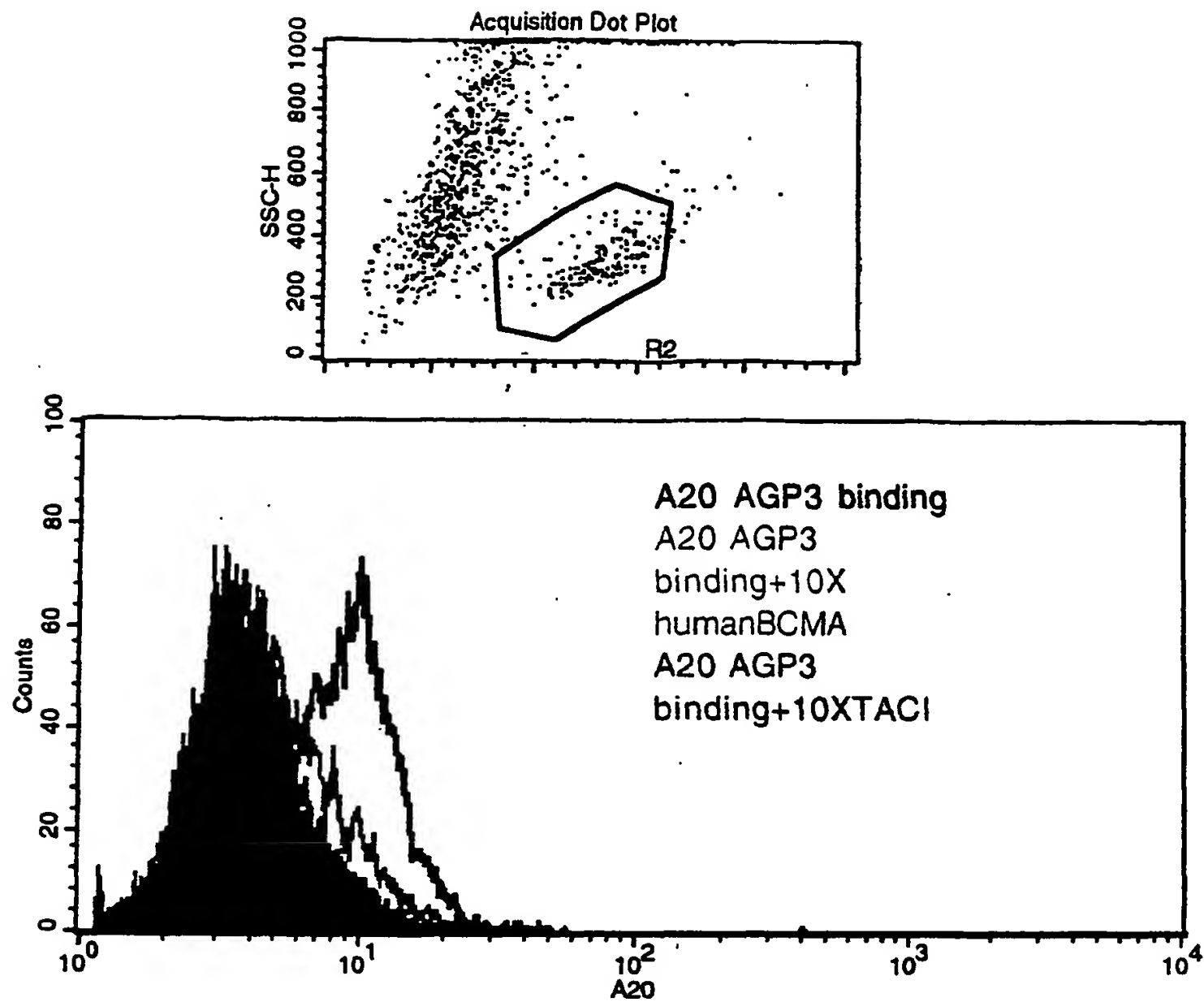


C.



Experiment
4-11-2000

25/48



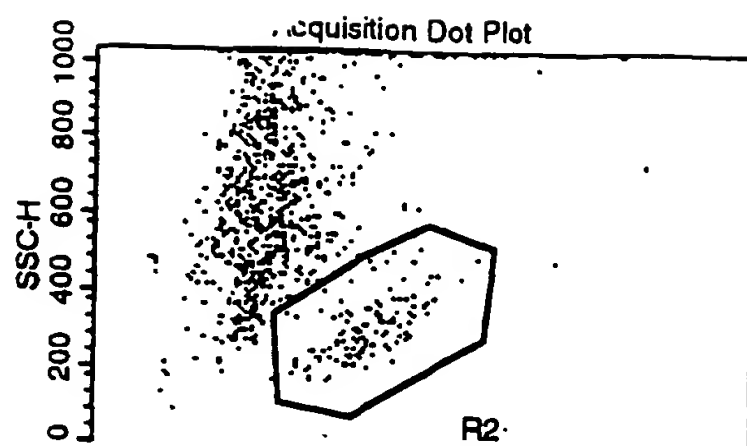
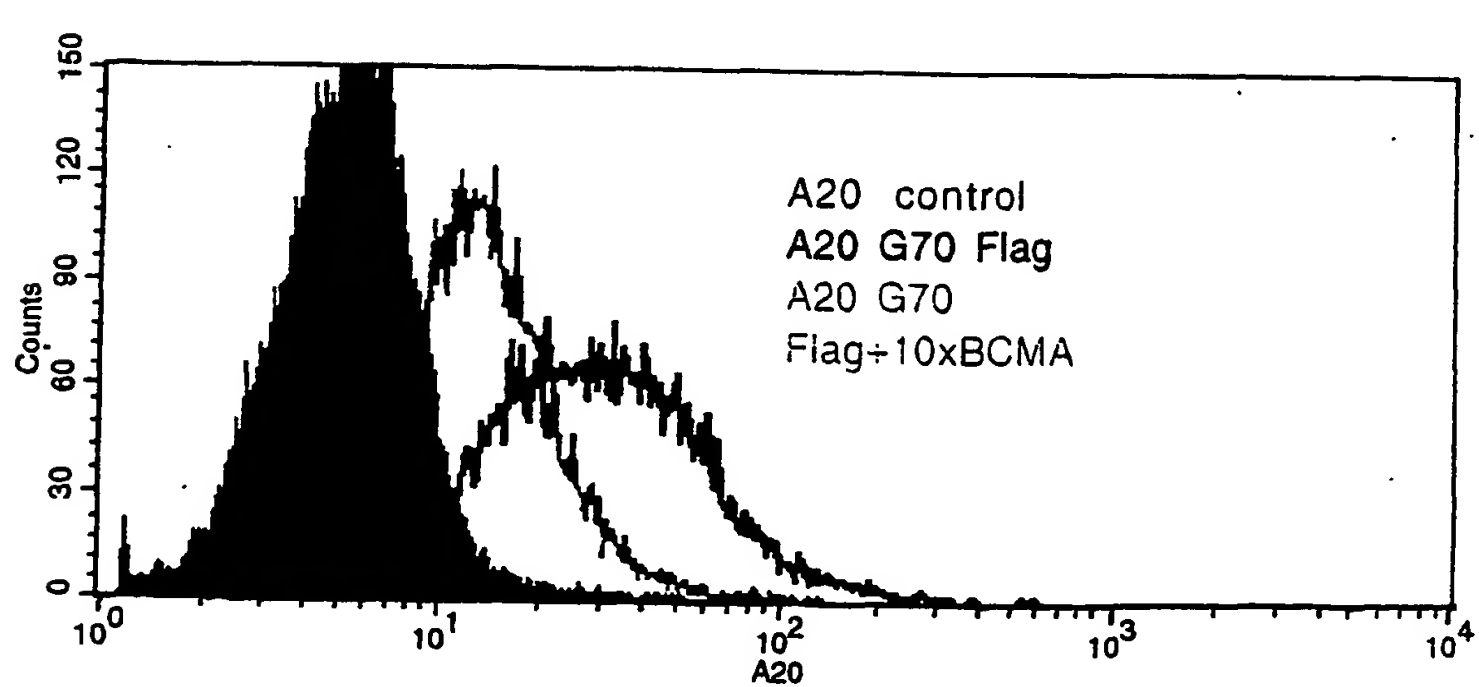
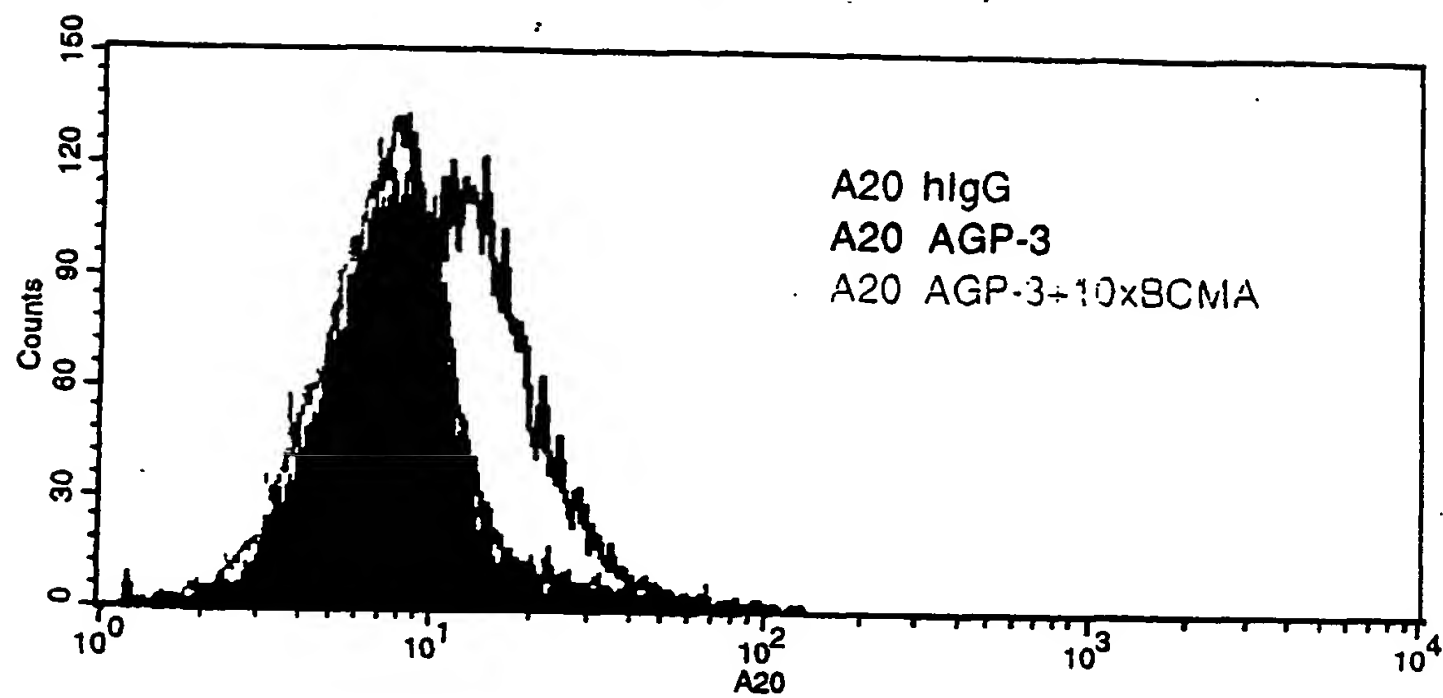
**Fig.19**

Fig.20

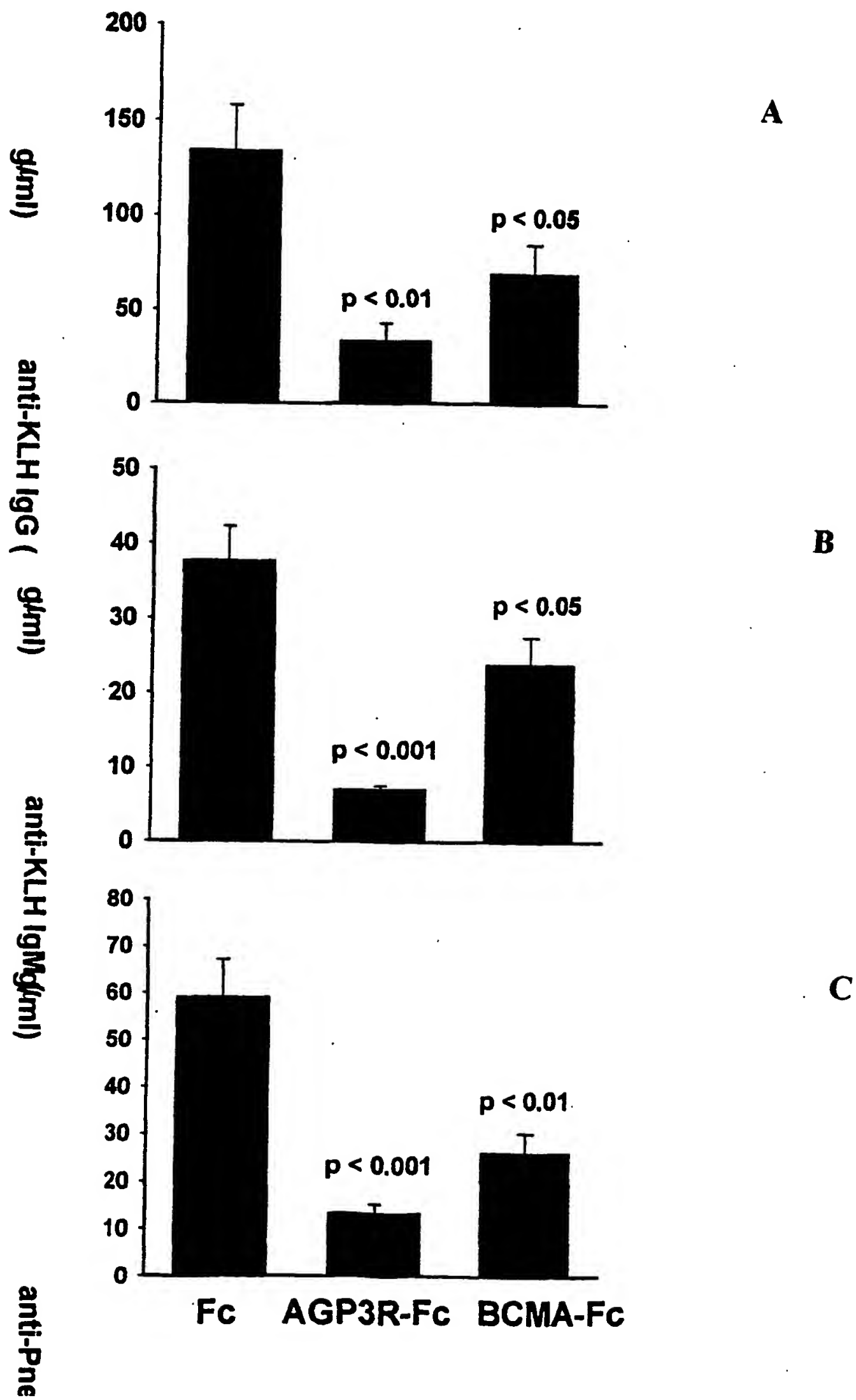


Figure 21 Fc-humanAPRIL

Fc-humanAPRIL protein sequence including the signal sequence, Fc domain, linker (XhoI site) and April:

1	MEWSWVFLFF	LSVTTGVHSD	KTHTCPPCPA	PELLGGPSVF
	LFPPKPKDTL			
51	MISRTPEVTC	VVVDVSHEDP	EVKFNWYVDG	VEVHNAKTKP
	REEQYNSTYR			
101	VVSVLTVLHQ	DWLNKEYKC	KVSNKALPAP	IEKTISKAKG
	QPREPQVYTL			
151	PPSRDELTKN	QVSLTCLVKG	FYPSDIAVEW	ESNGQPENNY
	KTPPPVLDSD			
201	GSFFLYSKLT	VDKSRWQQGN	VFSCSVMHEA	LHNHYTQKSL
	SLSPGKSRAV			
251	LTQKQKKQHS	VLHLVPINAT	SKDDSDVTEV	MWQPALRRGR
	GLQAQGYGVR			
301	IQDAGVYLLY	SQVLFQDVTF	TMGQVVSREG	QGRQETLERC
	IRSMPSHPDR			
351	AYNSCYSAGV	FHLHQGDILS	VIIPRARAKL	NLSPHGTFLG
	FVKL*			

Figure 22

**Fc-HumanAPRIL and soluble human AGP3
stimulate proliferation of primary B cells**

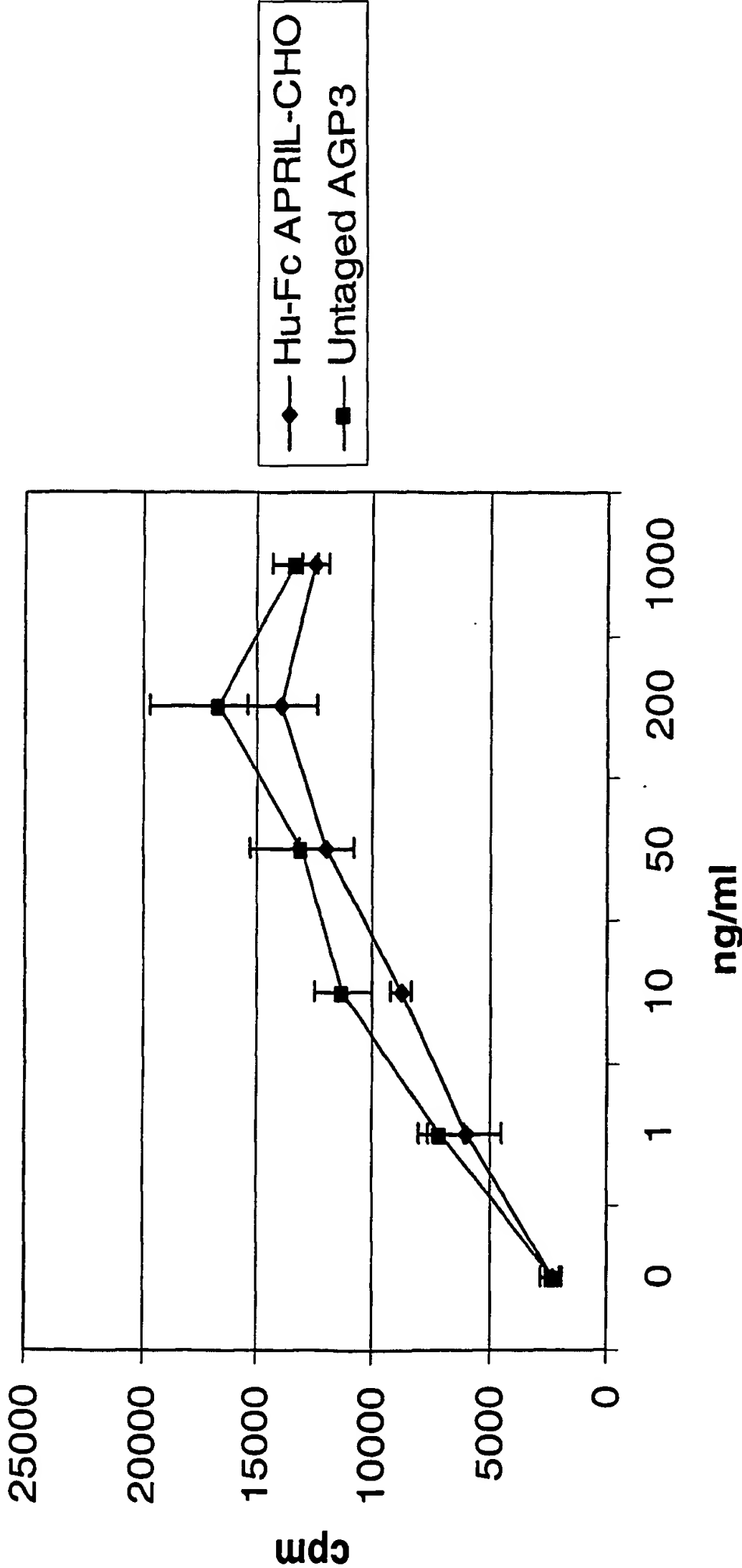


Figure 23

**hBCMA-Fc and wt hTACI-Fc inhibits
Flag-mAPRIL mediated mouse B cell
proliferation**

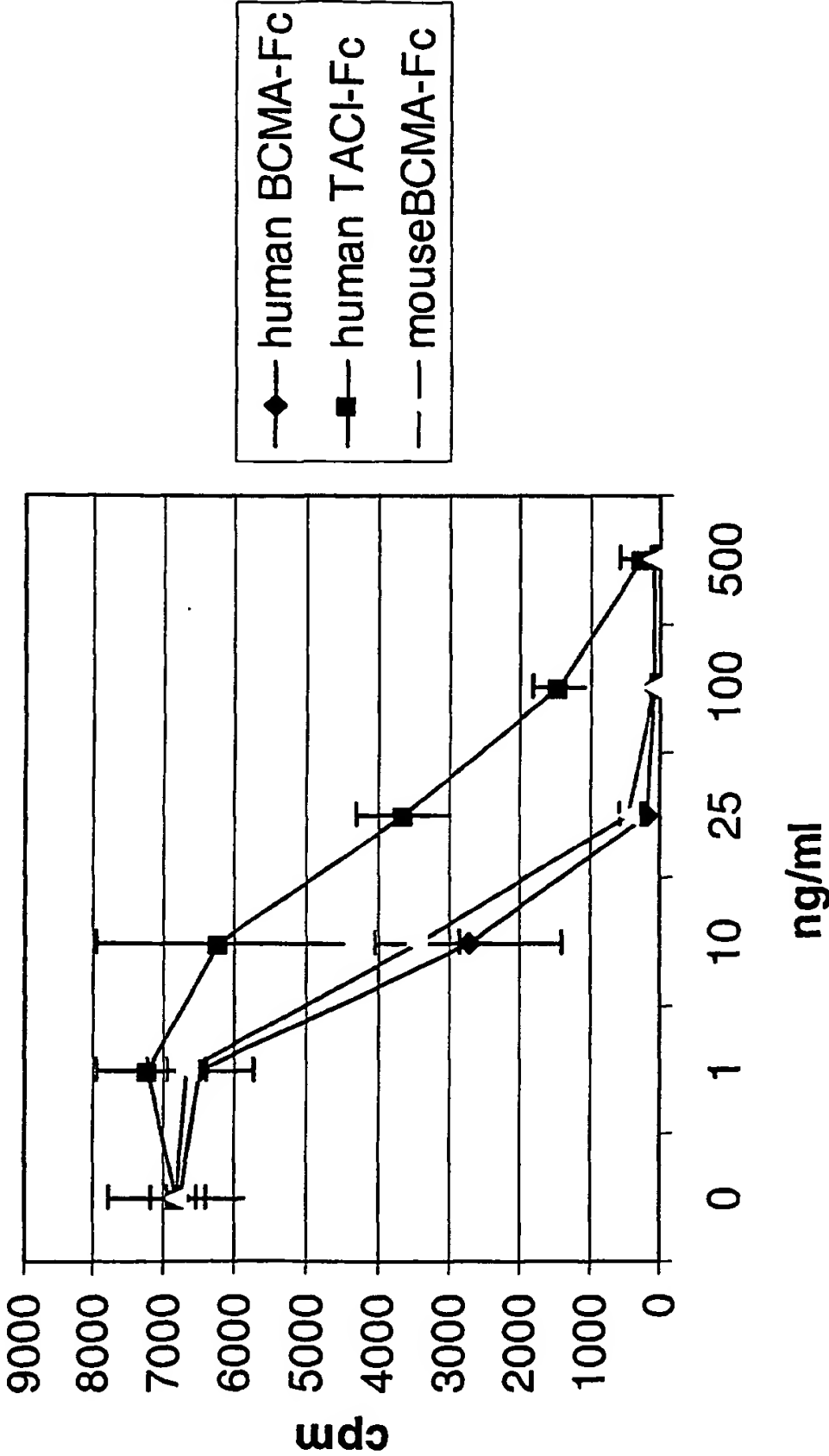


Figure 24:
hBCMA-Fc reduces PB B cell level *in vivo*
15 mg/kg ip on day 0, 3, and 6

BLOOD		WBC	#Lym	CD3+	CD3-B220+
		10e6/ml	10e6/ml	#	#
BCMA-Fc		5.30	3.81	2.3	1.3
	SD	0.39	0.43	0.32	0.27
	t test	0.03318	0.01570	0.24737	0.00506
Fc		8.02	6.43	2.7	3.2
	SD	1.27	1.52	0.6	0.6
Saline		6.90	5.55	2.1	2.9
	SD	2.04	1.79	0.5	1.2

Figure 25

hBCMA-Fc reduces spleen B cell levels *in vivo*
15 mg/kg ip on day 0, 3, and 6

spleen	WBC 10e6/ml	Lym (%)	spleen lym# 10ml(x10e6)	CD3-B220+ (%)	CD3-B220+ #
BCMA-Fc	9.12 SD 0.92	97.9 0.51	89.3 9.32	45.5 1.29	41.8 4.92
t test	0.02778	0.89118	0.02668	0.00234	0.02088
Fc	11.49 SD 1.62	97.9 0.38	112.5 15.65	50.6 1.95	57.1 9.67
Saline	11.48 SD 1.71	98.5 0.1	113.1 16.9	53.7 6.7	48.5 29.15

Figure 26

Flag-mAPRIL and hAGP3 mediated IgA production inhibited by hBCMA-Fc and hTACI-Fc *in vitro*

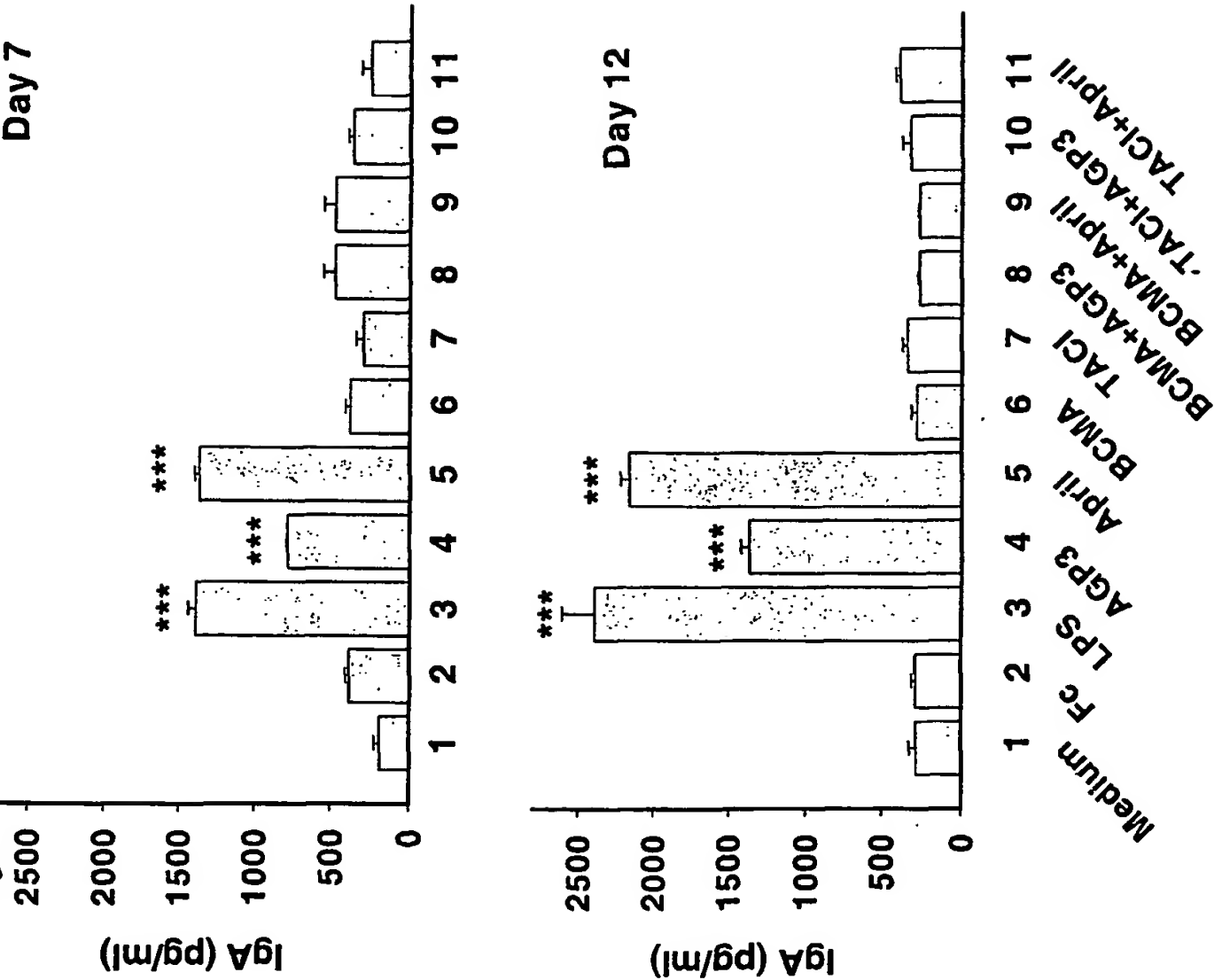


Figure 27
Flag-mAPRIL and hAGP3 Mediated IgG Production
Inhibited by BCMA-Fc and TACI-Fc *in Vitro*

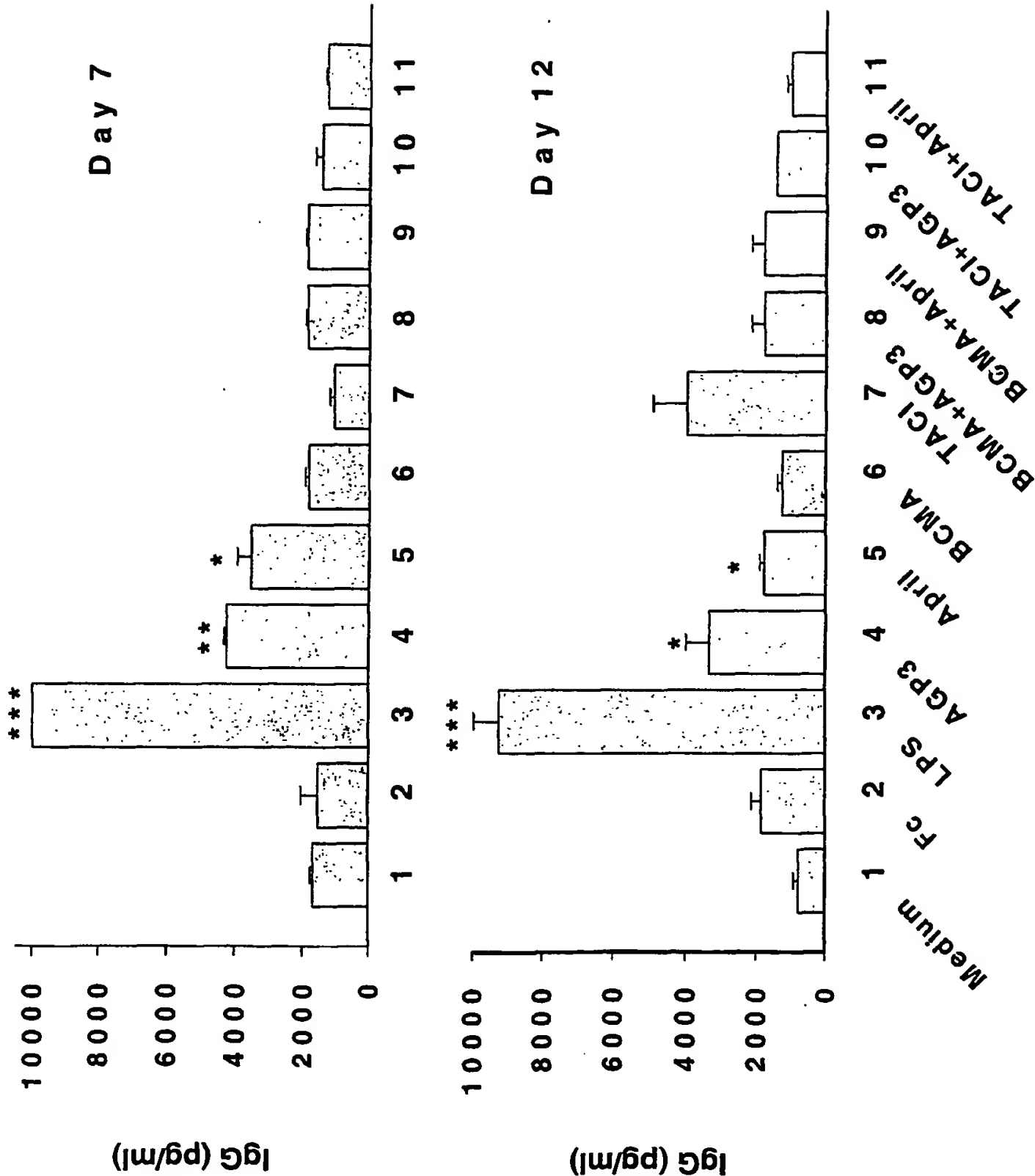


Figure 28: Significantly reduces total IgE and IgA in normal mice treated with mBCMA-Fc and trun hTACI-Fc 5 mg/kg ip day 0, 3, and 6

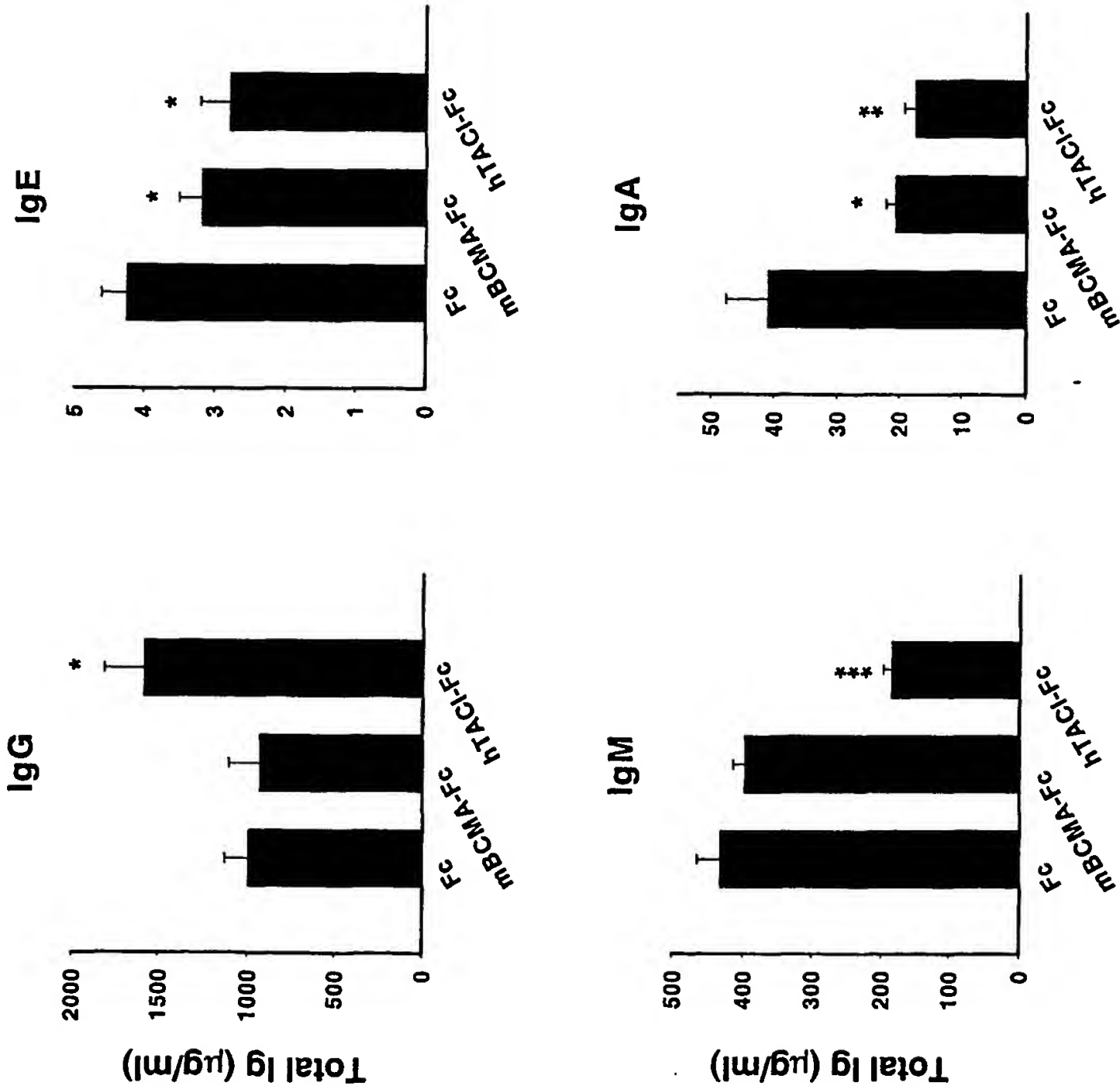
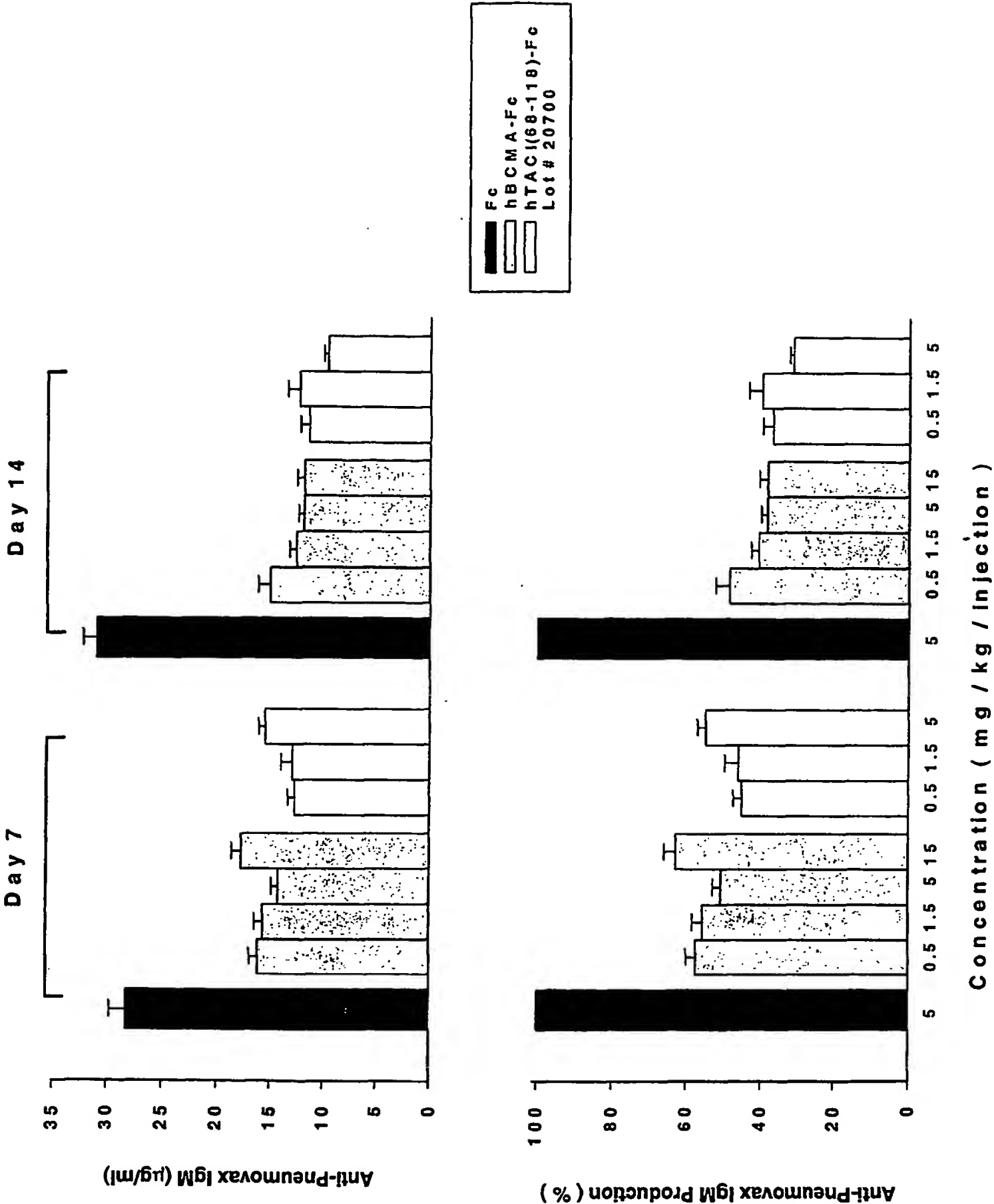


Figure 29: BCMA-Fc and truncated TACI-Fc at daily doses of 0.5 mg/kg inhibits humoral immunity *in vivo*



**Figure 30: Anti-mAPRIL c-19 MAb
inhibition of APRIL mediated B cell
proliferation**

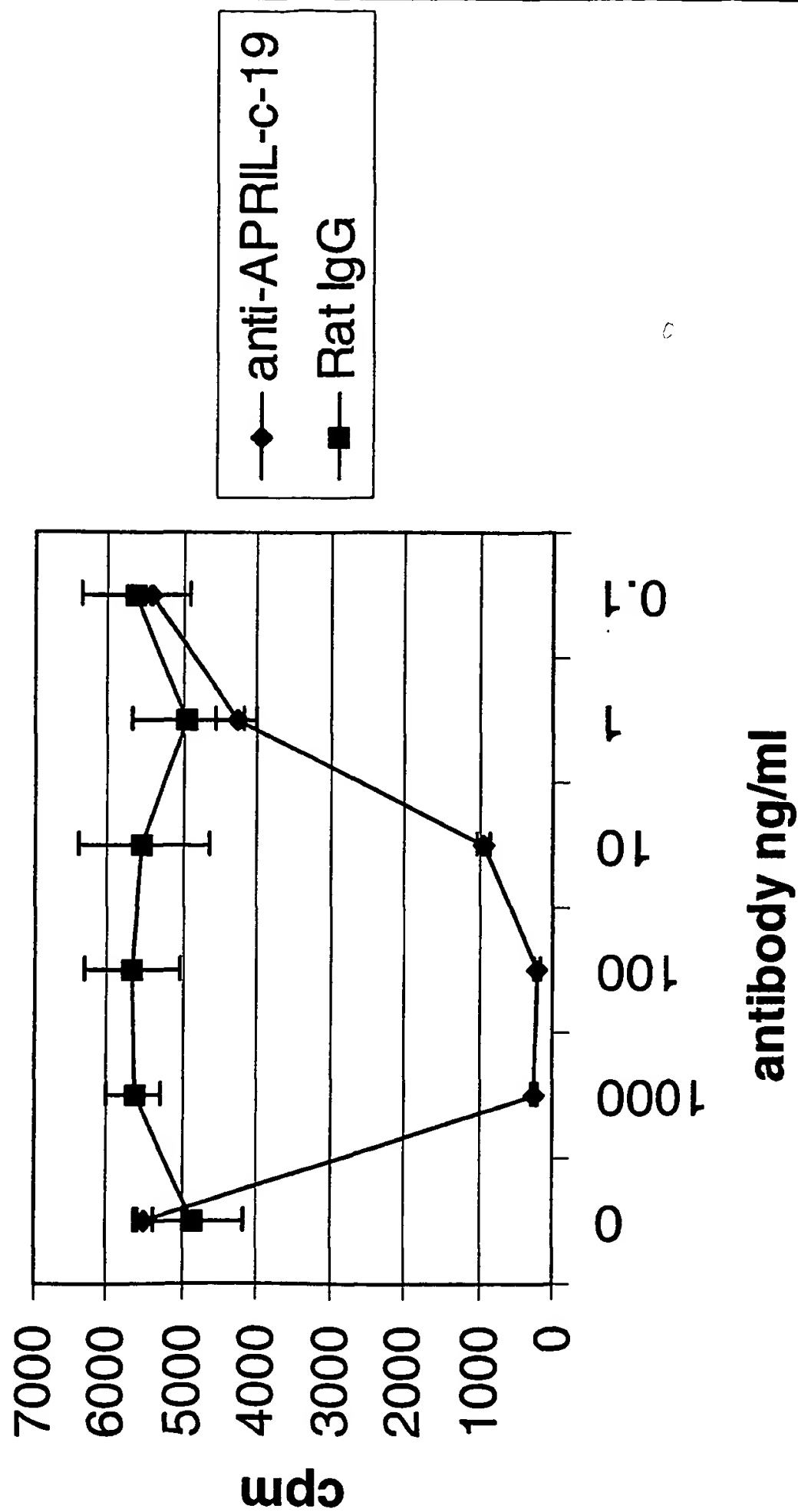
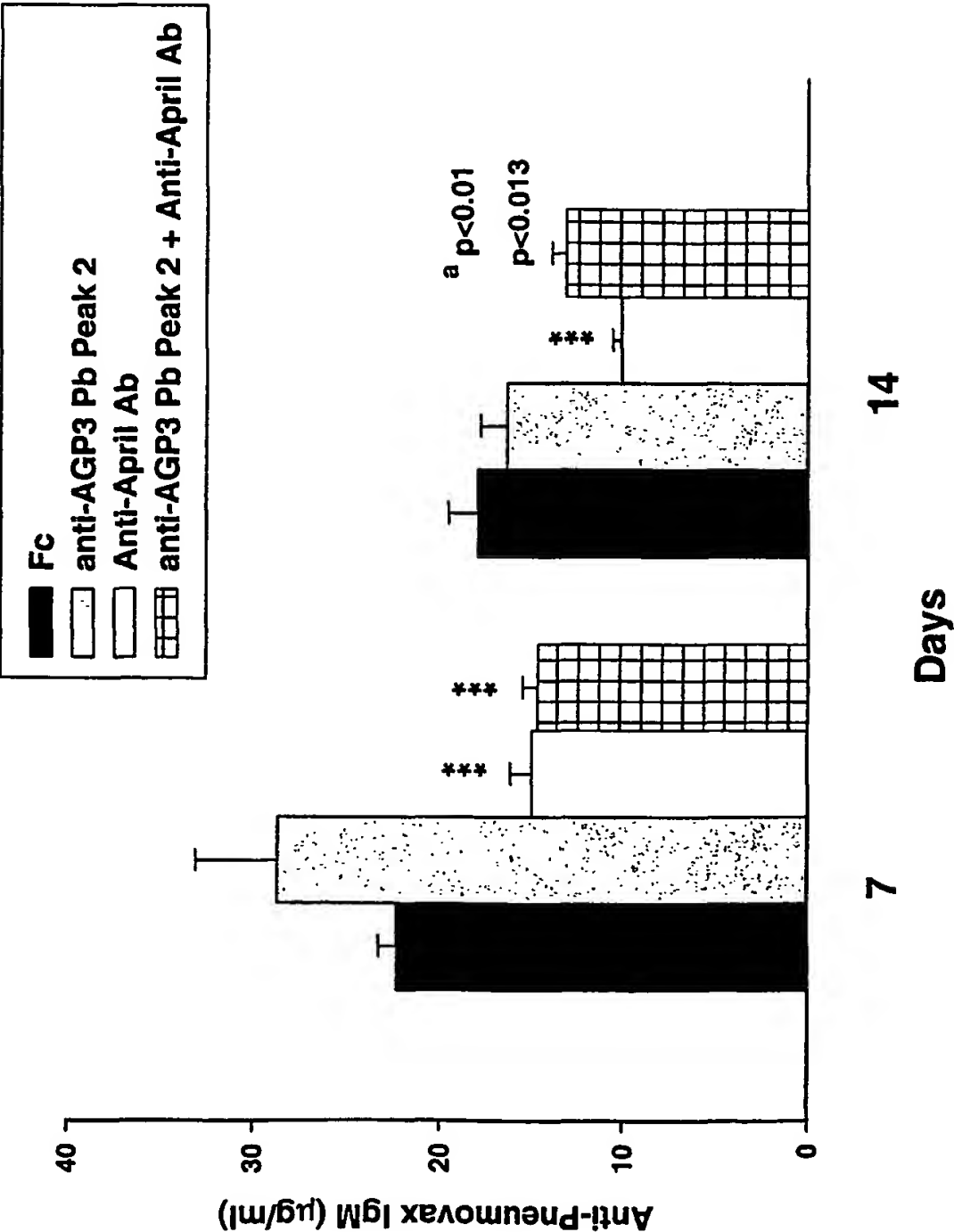


Figure 31
Neutralizing anti-mAPRIL Mab Reduces anti-Pneumovax IgM *In Vivo*
5 mg/kg ip on day 0, 3, and 6

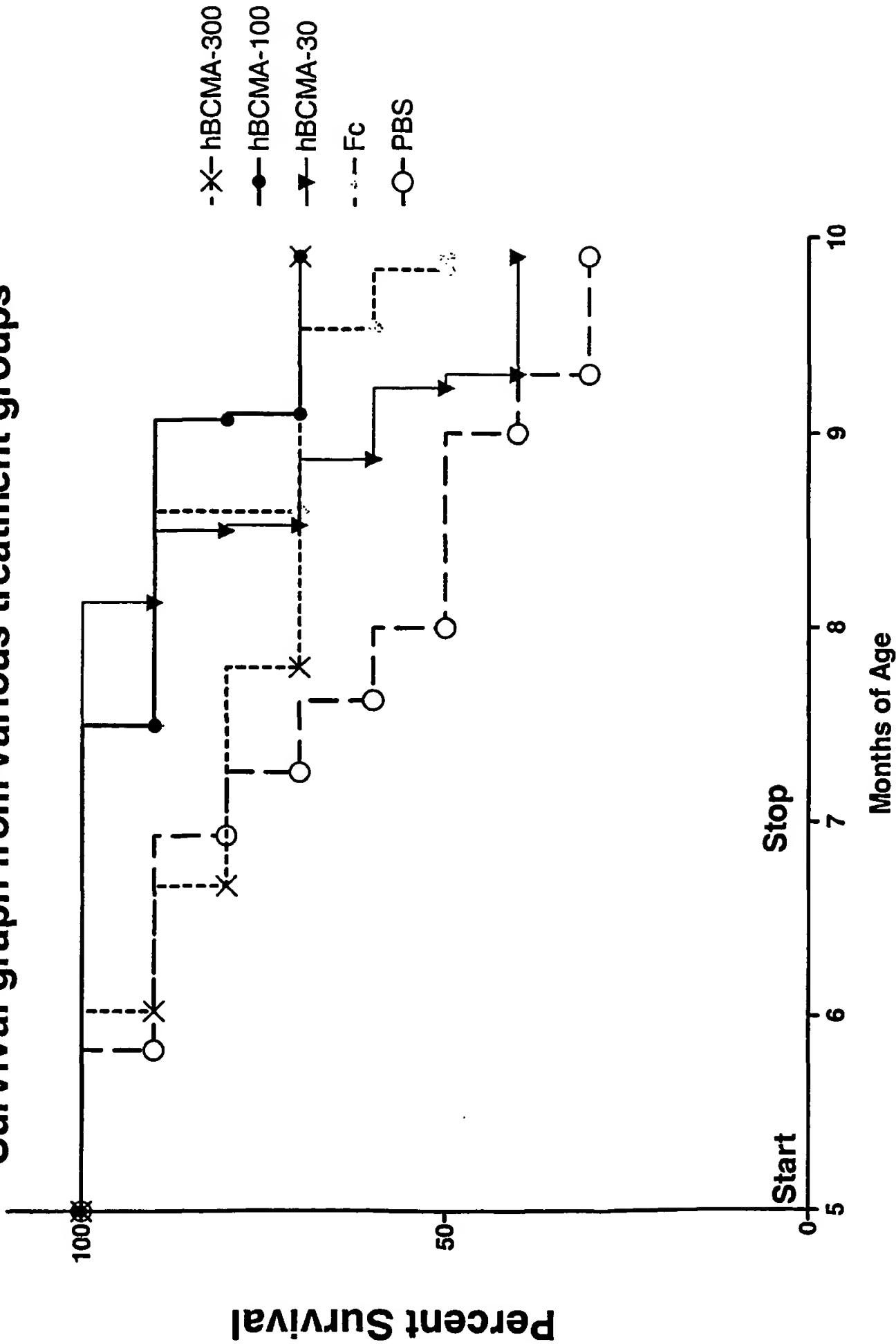


^a difference between Anti-April Ab and anti-AGP3 Pb Peak 2+ Anti-April Ab Groups

12.15.00 lupus exp.

Figure 32: Effect of hBCMA-Fc in NCB/NCWF1 mice

Survival graph from various treatment groups

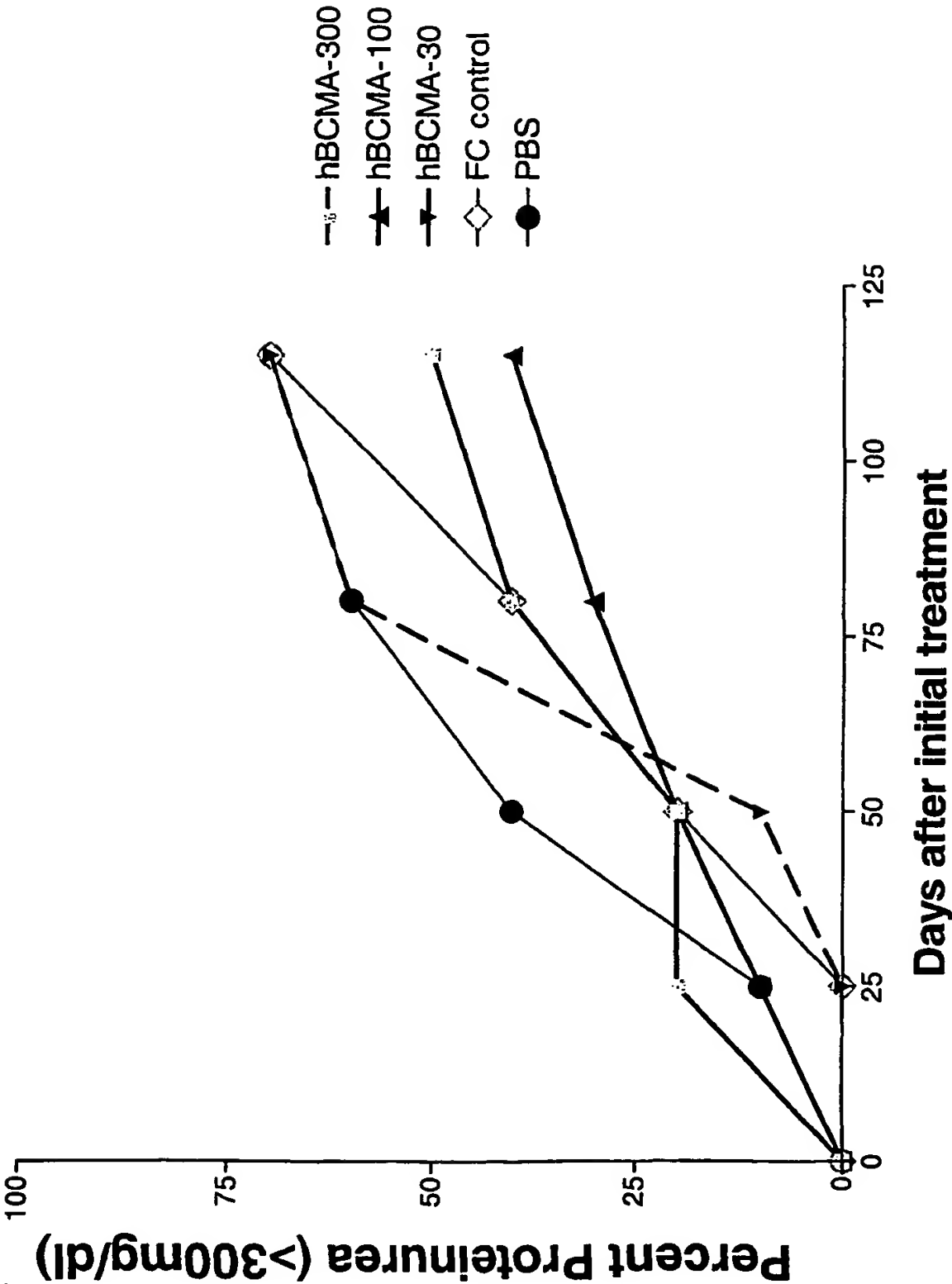


N=10 Mice were treated for 8 weeks 3x/week with the indicated proteins. KIN2 group had 12 mice. The 100 in the legend stands for 100 µg of protein or 4mg/kg i.p.

12.15.00 lupus exp

Figure 33: Effect of hBCMA-Fc in NCB/NCWF1 mice

Percentage of mice with proteinurea (>300mg/dl)
from various treatment groups



N=10 Five month old BW F1 mice were treated with protein for 8 weeks i.p.
The hBCMA-300 stands for hBCMA-fc 300µg/mouse (12mg/kg)

Figure 34: Analysis of antibodies to dsDNA from the peripheral blood from various treatment groups of BWF1 at day 0,30,60, and 90.

MEAN anti-dsDNA isotypes in U/ml								
Group #	Day 0		Day 30		Day 60		Day 90	
	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
hBCMA-300	179	560	163	371	150	706	171	841
hBCMA-100	150	430	259	718	171	822	339	1031
hBCMA-30	377	592	297	458	401	664	424	601
FC.	149	371	234	283	384	331	432	351
PBS	308	292	439	311	247	576	720	467

Standard Deviation of the above means

Group #	Day 0		Day 30		Day 60		Day 90	
	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
hBCMA-300	104	303	116	211	62	518	62	734
hBCMA-100	109	262	306	461	212	758	371	1225
hBCMA-30	363	455	281	430	305	606	421	400
FC.	68	160	150	93	391	151	233	237
PBS	311	73	474	152	247	370	870	327

Figure 35: Evaluation of B cell numbers at treatment day 60 from the 12mg/kg (30 ug), 4mg/kg (100ug), and 1.3mg/kg (300 ug) dose of hBCMA-Fc groups along with the Fc and PBS control groups.

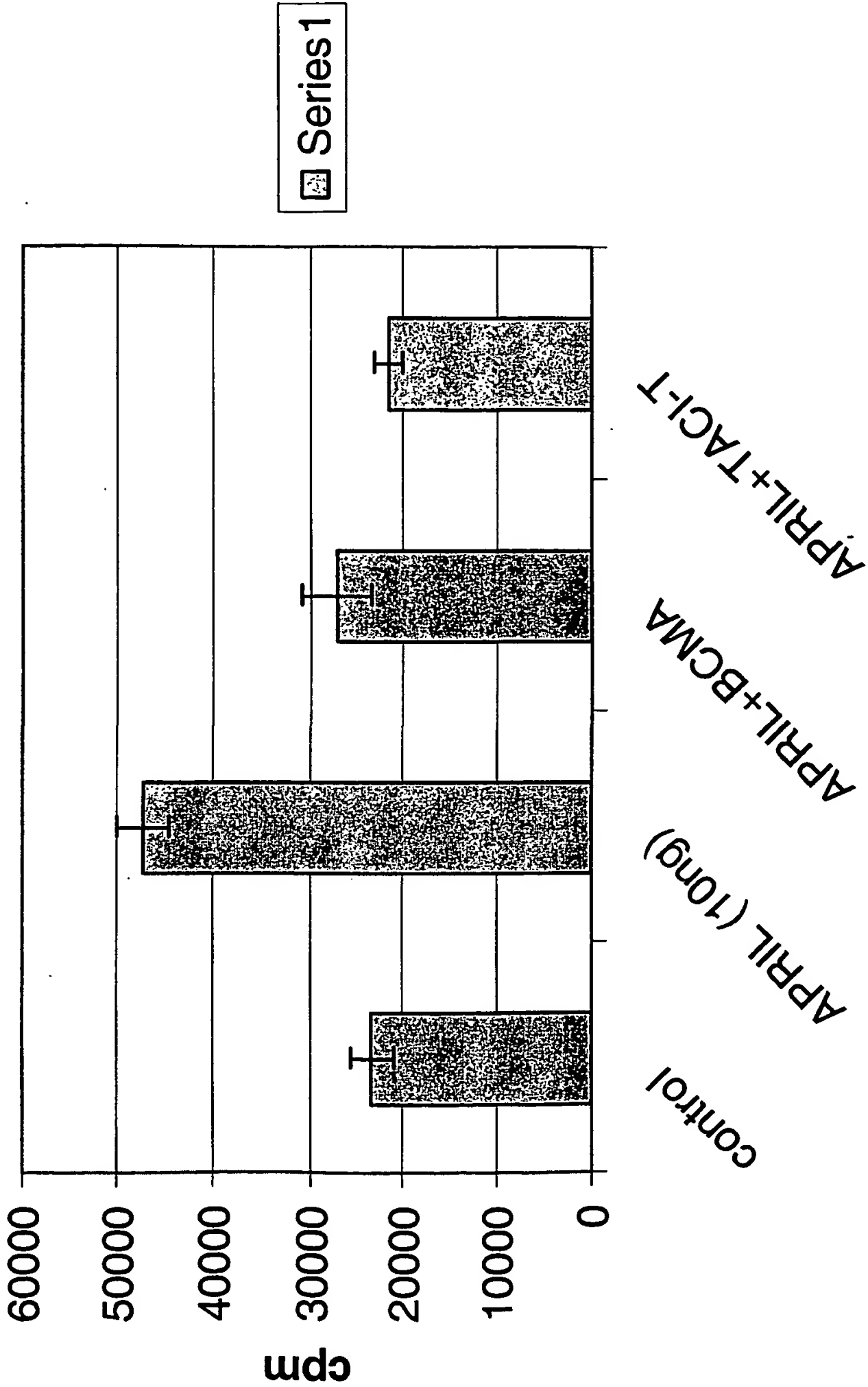
hBCMA-fc-300				hBCMA-100				hBCMA-FC-30			
Mouse#	%CD4	%CD8	%B220	%CD4	%CD8	%B220		%CD4	%CD8	%B220	
1.0	16.3	11.0	16.4	26.1	14.9	10.1		9.0	6.9	10.3	
2.0	24.1	11.1	11.6	21.1	11.3	10.6		10.0	5.2	23.4	
3.0	18.2	7.4	9.9	24.6	13.3	8.3		11.0	6.4	29.2	
4.0	25.4	13.3	13.1	20.0	11.3	13.4		12.0	7.6	31.5	
x	21.0	10.7	12.8	23.0	12.7	10.6		x	6.5	23.6	
sd	4.4	2.4	2.8	2.9	1.7	2.1		sd	1.0	9.5	
Fc				PBS							
33.0	7.0	8.1	25.4	16.9	8.3	15.5					
34.0	10.7	4.9	15.3	19.1	12.1	19.5					
35.0	18.9	9.3	21.0	7.1	3.4	17.5					
36.0	20.1	11.1	21.0	19.9	11.4	26.5					
x	14.2	8.4	20.7	15.8	8.8	19.8					
sd	6.4	2.6	4.1	5.9	4.0	4.8					

**Figure 36: Specific APRIL binding to Human Cell lines determined
by FACS analysis**

APRIL binding

HT 29 Colon adenocarcinoma	++ +
NCI 460 Lung carcinoma	++ +
PC3 Prostate adenocarcinoma	++
C6 Glial carcinoma	++
Raji Burkitt lymphoma	++ +
A20 Mouse B cell lymphoma	++ +
U266BI Myeloma	++ +
A435 Epidermoid carcinoma	--
A469 Kidney carcinoma	--
MDA-231 breast adenocarcinoma	--

Figure 37: Effect of APRIL, BCMA-Fc and TACI-Fc truncated on U266BI cell proliferation



Gang Yu, Theill lab 010426

**Figure 38: APRIL and AGP3 stimulates
and BCMA-Fc inhibits B lymphoma cell
proliferation**

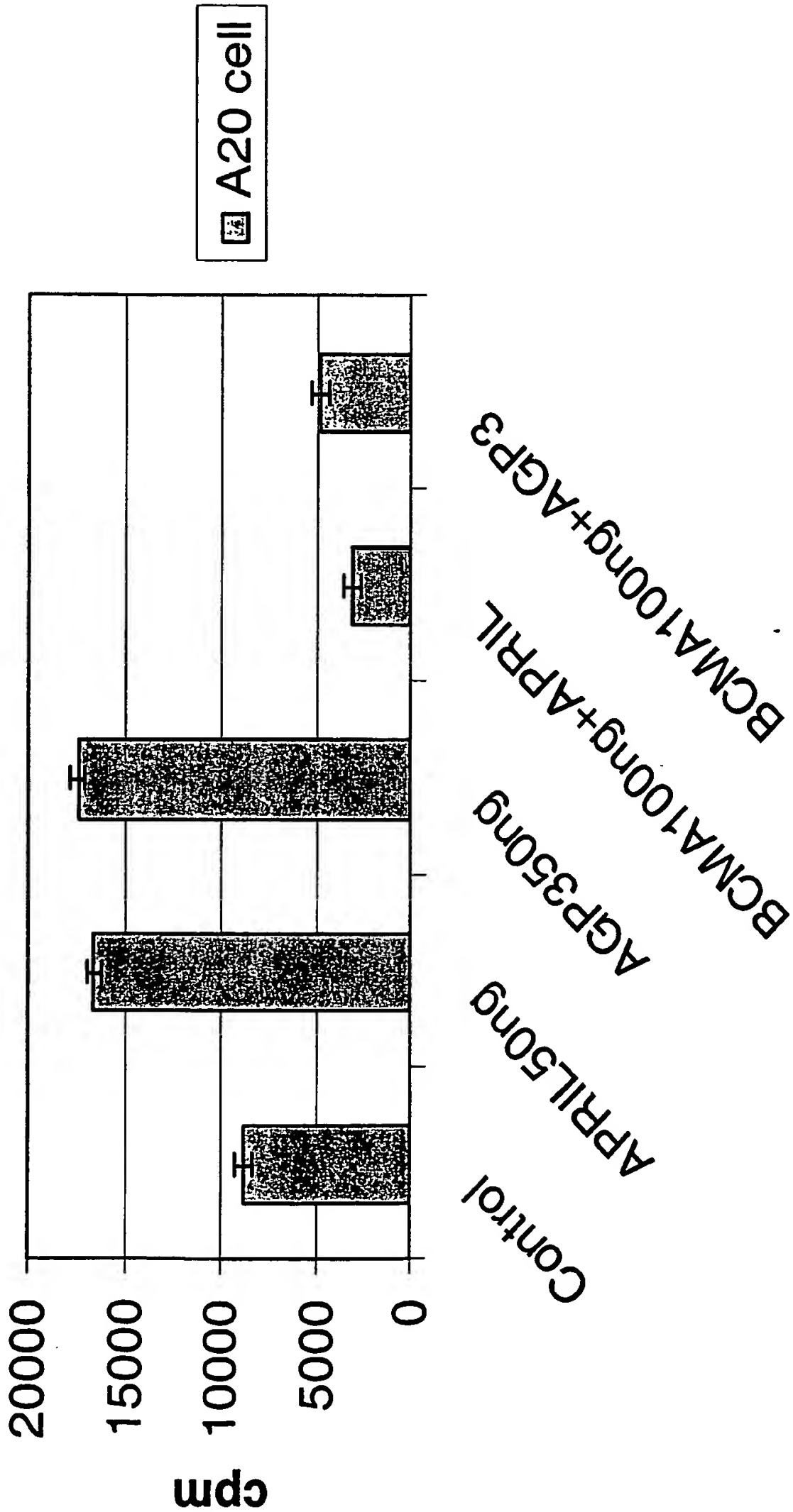


Figure 39: Effects of BCMA & hTACI on the Growth of A20 in Balb/c Mice

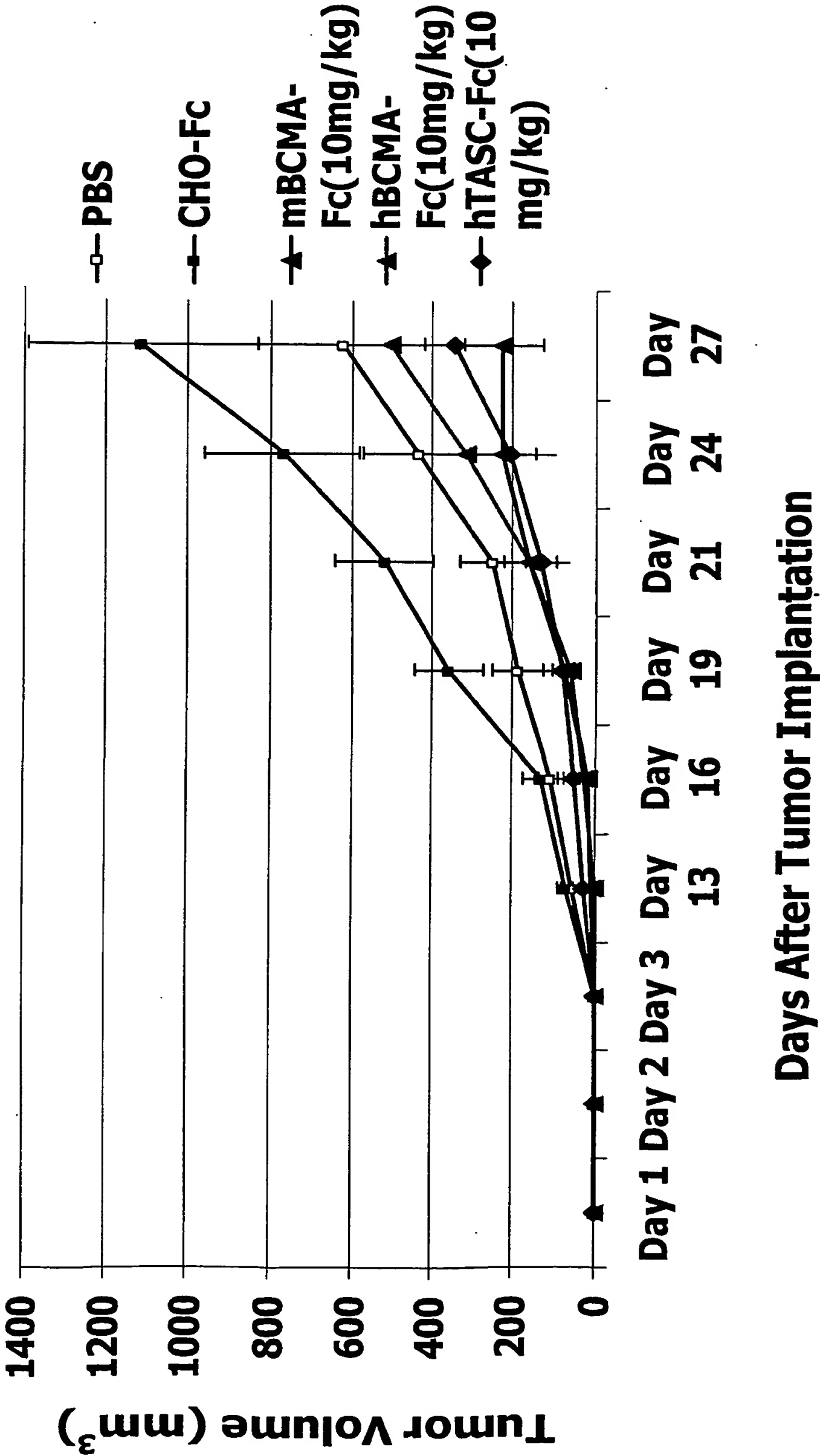
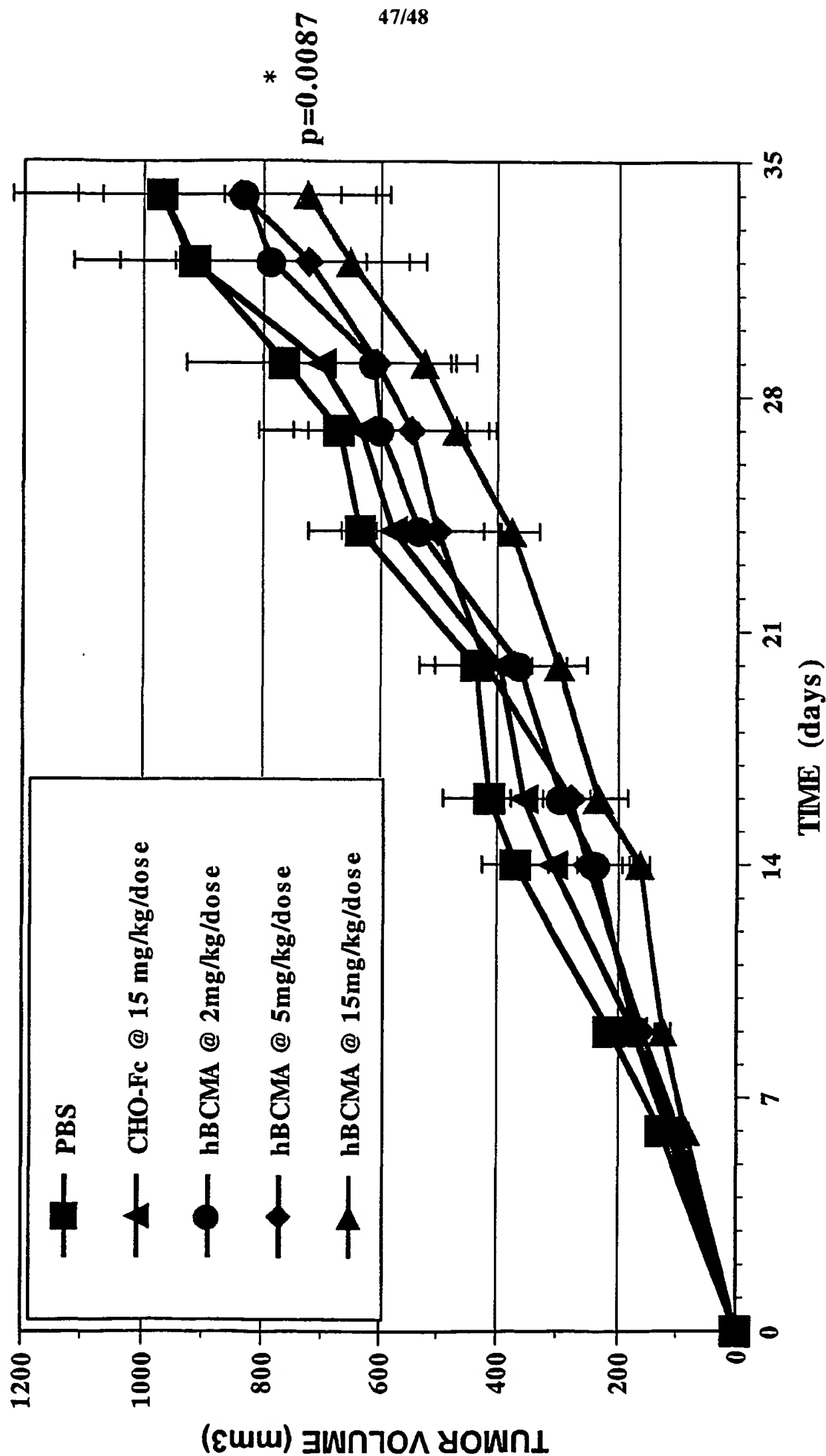
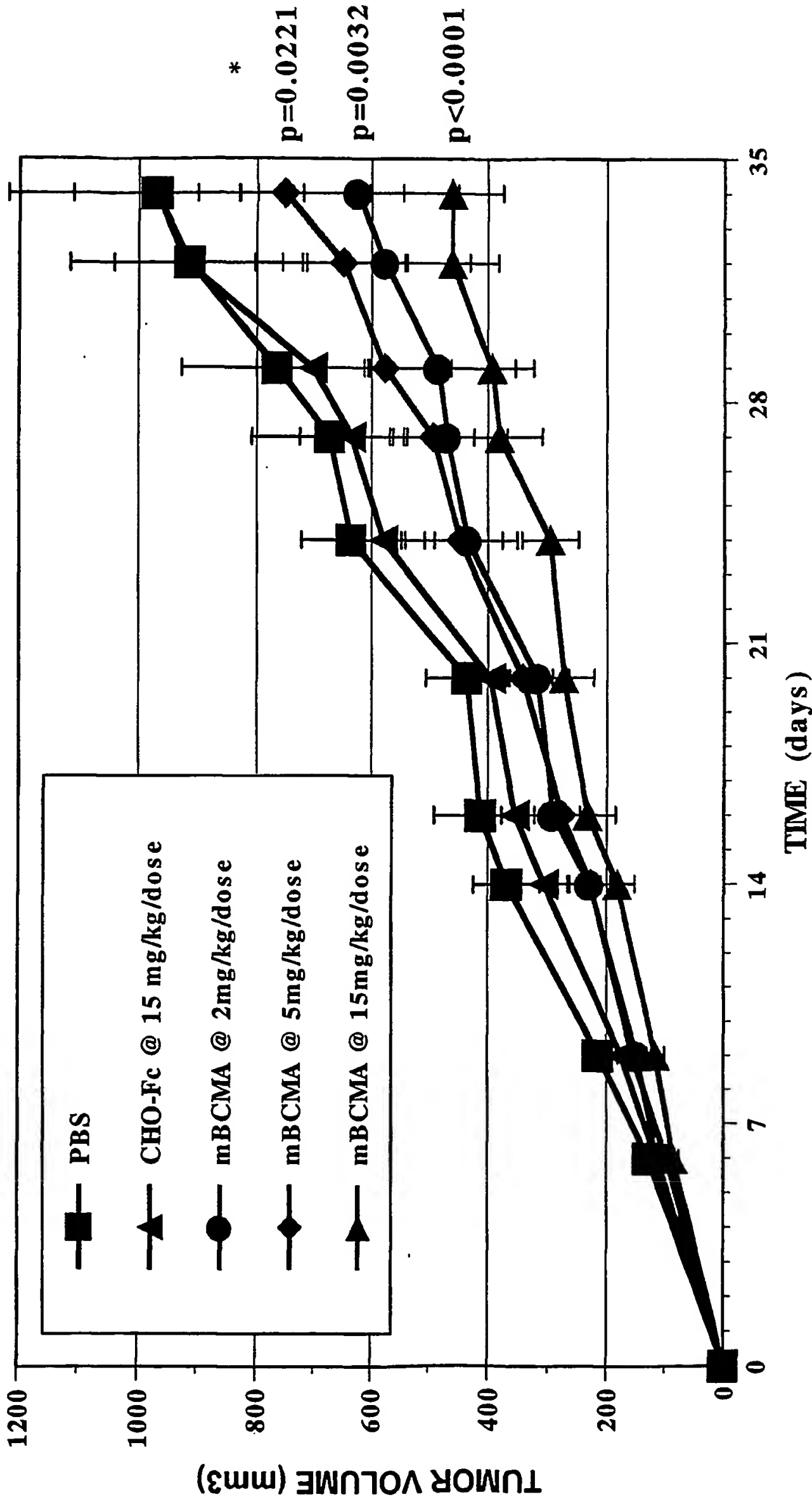


Figure 40
EFFECT OF HUMAN BCMA-Fc AGAINST HT-29 SC TUMOR GROWTH
Rx: IP, Q2D, day 0



* Linear growth ANOVA with Dunnett's correction for multiple testing (n=10/group)

Figure 41
EFFECT OF MURINE BCMA-Fc AGAINST HT-29 SC TUMOR GROWTH
Rx: IP, Q2D, day 0



* Linear growth ANOVA with Dunnett's correction for multiple testing (n=10/group)

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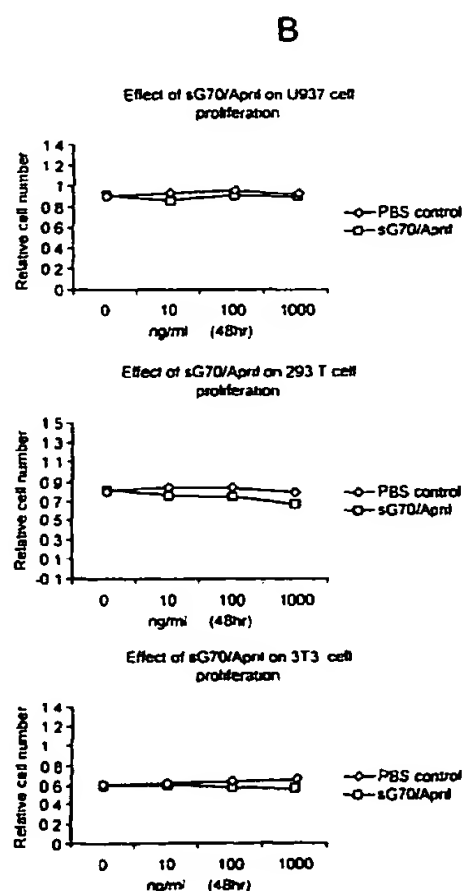
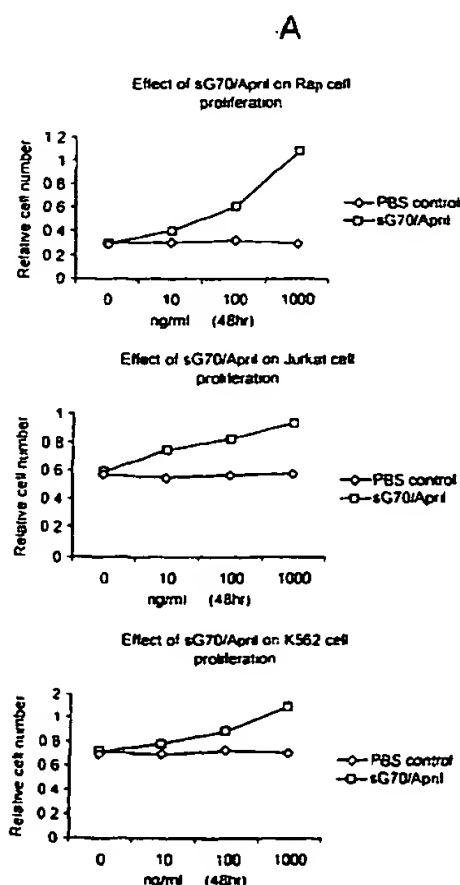
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[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS OF MATTER CONCERNING APRIL/G70, BCMA, BLYS/AGP-3, AND TACI



(57) Abstract: This invention concerns interactions among APRIL/G70, AGP-3/BLYS, BCMA, and TACI and related methods of use and compositions of matter. It has been found that (1) sAPRIL/G70 binds to the cell-surface receptors BCMA and TACI on T and B lymphoma cells, resulting in stimulation of proliferation of primary human and mouse B and T cells both *in vitro* and *in vivo*; (2) APRIL competes with AGP3's binding to TACI and BCMA; (3) sBCMA inhibits APRIL and AGP3 binding to its receptors; (4) sBCMA ameliorates T cell dependent and T cell independent humoral immune responses *in vivo*; (5) sTACI inhibits APRIL and AGP3 binding to its receptors and ameliorates T cell dependent and T cell independent humoral immune responses *in vivo*; and (6) BCMA exhibits similarity with TACI within a single cysteine rich domain located N-terminal

to a potential transmembrane domain. These discoveries provide a strategy for development of therapeutics for treatment of autoimmune diseases, and cancer, for prevention of transplant rejection. Disease states and disease parameters associated with APRIL and AGP-3 may be affected by modulation of BCMA or TACI; disease states and parameters associated with TACI can be affected by modulation of APRIL; disease states and parameters can be affected by modulation of any of TACI, BCMA, APRIL and AGP-3 by a single therapeutic agent or two or more therapeutic agents together.



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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, GENSEQ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 39361 A (ST JUDE CHILDRENS RES HOSPITAL) 11 September 1998 (1998-09-11) page 24, line 20 -page 25, line 28 page 49, line 22-32 page 58, line 1-22	1,3,7, 11-17, 21-29, 31-37
Y		9,10, 18-20,30
X	WO 99 12965 A (TSCHOPP JURG ;BIOGEN INC (US)) 18 March 1999 (1999-03-18) cited in the application page 15, line 40 -page 16, line 9 example 2 figure 3	5,6, 14-17, 21, 27-29, 31-37

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/15504

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>HAHNE M ET AL: "APRIL, a new ligand of the tumor necrosis factor family, Stimulates tumor cell growth" JOURNAL OF EXPERIMENTAL MEDICINE, TOKYO, JP, vol. 188, 21 September 1998 (1998-09-21), pages 1185-1190, XP002093077 ISSN: 0022-1007 cited in the application page 1187, right-hand column -page 1189, left-hand column</p> <p>---</p>	6,14-17, 27-29, 31-37
Y	<p>CUBILLOS SUZANA ET AL: "Effect of blocking TNF on IL-6 levels and metastasis in a B16-BL6 melanoma/mouse model." ANTICANCER RESEARCH, vol. 17, no. 3C, 1997, pages 2207-2211, XP001051935 ISSN: 0250-7005 page 2210, left-hand column; figure 5</p> <p>---</p>	9,10
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A	<p>GROSS JANE A ET AL: "TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease." NATURE (LONDON), vol. 404, no. 6781, 27 April 2000 (2000-04-27), pages 995-999, XP002140939 ISSN: 0028-0836 cited in the application the whole document</p> <p>---</p>	1-37
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P,X	<p>WO 00 40716 A (ZymoGenetics Inc) 13 July 2000 (2000-07-13) page 2, line 15-35; claims 1-8,20,29-38</p> <p>---</p> <p>-/--</p>	1,3,5,7, 8,11-14, 22-26

INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 01/15504

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JEFFREY S THOMPSON ET AL: "BAFF binds to the tumor necrosis factor receptor-like molecule B cell maturation antigen and is important for maintaining the peripheral B cell population" JOURNAL OF EXPERIMENTAL MEDICINE, TOKYO, JP, vol. 192, no. 1, 3 July 2000 (2000-07-03), pages 129-135, XP002156772 ISSN: 0022-1007 figures 3,4; table I ---	3,7,14, 22-29, 31-37
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E	WO 01 60397 A (GENENTECH INC) 23 August 2001 (2001-08-23) page 6, line 38 -page 7, line 23 examples 1-11 ---	27-29, 31-37
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INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 01/15504

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	<p>LI S ET AL: "A peptide antagonist mimicking the 3rd TNF-like cysteine-rich domain of mouse OPG inhibits RANKL/M-CSF induced osteoclastogenesis." JOURNAL OF BONE AND MINERAL RESEARCH, vol. 16, no. Suppl. 1, September 2001 (2001-09), page S209 XP001058389 Twenty-Third Annual Meeting of the American Society for Bone and Mineral Research; Phoenix, Arizona, USA; October 12-16, 2001 ISSN: 0884-0431 abstract</p> <p>-----</p>	7-10, 14, 15, 19-26

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-6, 14-21, 27-37 (partially)

Present claims 1, 2 relate to methods of inhibiting B or T-cell proliferation or of inhibiting APRIL activity by using certain compounds defined by reference to a desirable characteristic or property, namely that they are antagonists of the TNF receptor-like molecules TACI or BCMA.

The claims cover all methods using compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the method by the compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the methods which comprise the use of anti-TACI or anti-BCMA antibodies (as mentioned on pages 24-36 of the description and on claims 11-13).

Present claims 3, 4, 14-17, 19-21 and 27-37 relate to a method of inhibiting TACI and/or BCMA activity by administering a compound and to compositions of matter comprising a peptide, wherein the compound and the peptide are defined by reference to a desirable characteristic or property, namely, that they are specific binding partners for APRIL and/or AGP-3.

The claims cover all methods using compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the method by the compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the methods which comprise the use of extracellular or consensus region of the TNF receptor-like molecules TACI and BCMA, as mentioned on page 37 (lines 9-22), on pages 67-71 of the description and on claims 7-9, 22, 25.

Present claims 5, 6, 14-16, 18, 19, 21 and 27-37 relate to methods of treatment of different diseases by administering an agent and to compositions of matter comprising a peptide sequence, wherein both the

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

agent and the peptide sequence are defined by reference to a desirable characteristic or property, namely, that they are specific binding partners for TACI or BCMA.

The claims cover all methods using compounds and compositions of matter comprising peptides having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the method and the composition of matter by the compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the methods which comprise the use of APRIL as a binding partner for TACI and/or BCMA as mentioned on page 65 line 26 to page 67 line 27.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

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